PATENT
Customer No. 22,852
Attorney Docket No. 06478.1507-00

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	)	
	)	
Reinhard BOLLI *et al.*	)	Group Art Unit: 1644
	)	
Application No.: 10/579,357	)	Examiner: Yunsoo Kim
	)	
PCT filed: November 17, 2004	)	
§ 371 date: May 16, 2006	)	Confirmation No.: 2138
	)	
For: IMMUNOGLOBULIN	)	
PREPARATIONS HAVING	)	**VIA EFS WEB**
INCREASED STABILITY	)	

Attention: Mail Stop Appeal Brief-Patents
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

APPEAL BRIEF UNDER BOARD RULE § 41.37

In support of the Notice of Appeal filed September 7, 2011, and further to Board Rule 41.37, Appellant presents this brief and files herewith the fee of $620.00 required under 37 C.F.R. § 1.17(c).

This Appeal responds to the June 7, 2011, rejection of pending, elected claims 29-45.

If any additional fees are required or if the enclosed payment is insufficient, Appellant requests that any additional required fees be charged to Deposit Account No. 06-0916.

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Real Party In Interest

CSL Behring AG is the real party in interest. (See the assignment documents recorded on August 16, 2006, at Reel 018126, Frame 0880, and on April 23, 2008, at Reel 020841, Frame 0702.)

Related Appeals and Interferences

There are currently no other appeals or interferences, of which Appellant, Appellant's legal representative, or Assignee are aware, that will directly affect or be directly affected by or have a bearing on the Board's decision in this appeal.

Status Of Claims

Claims 18, 20-21, and 29-45 are currently pending in the application. (*See* Office Action of June 7, 2011, at 2; Request for Continued Examination and Reply to Office Action under 37 C.F.R. § 1.114 of September 17, 2010.) Claims 29-45 are currently under examination, while claims 18, 20, and 21 were previously withdrawn. (*See* Office Action of June 7, 2011, at 2; Office Action of September 26, 2008, at 2; Restriction Requirement of May 17, 2008.) Hence, only claims 29-45 are subject to this appeal.

Claims 1-17 and 22-28 were previously cancelled without prejudice or disclaimer. (*See* Reply to Office Action under 37 C.F.R. § 1.114 of September 17, 2010, Amendments to the Claims.)

Status Of Amendments

All prior amendments have been entered.

Summary Of Claimed Subject Matter

All of the claims relate to a stable "polyclonal IgG preparation." Polyclonal IgG preparations were known in the art as of the filing date of this application and were, for example, commercially available as therapeutics. As described in a 1994 review by Tankersley, "[v]irtually all therapeutic immune globulins (Ig) [polyclonal IgG preparations] are prepared from large pools of human plasma by a process of selective precipitation with ethanol . . ." (*Immunological Reviews* 139: 159-172, at 160, first full paragraph; entered into the record on June 2, 2011; copy attached.) Tankersley also comments that polyclonal IgGs were often made into preparations for intravenous pharmaceutical use. (*See Id.* at 159.) The review goes on to comment that "[t]he regulatory agencies of most countries require that each lot of Ig, or Igi.v. [polyclonal IgG preparation], shall be prepared from a pool of approximately equal amounts of plasma from not less than 1000 donors" but that "this requirement also affects the potential for the Igi.v. to contain anti-idiotypes recognizing other antibodies present either in the Igi.v. itself or in recipients." (*Id.* at 161, first full paragraph.) The review then explains that such idiotype – anti-idiotype recognition leads to a characteristic dimerization problem in polyclonal IgG preparations. (Tankersley at 161-163.)

Another review by Lemm, published in 2002, provides a general introduction to polyclonal IgG preparations, and describes how they were typically prepared from precipitated, pooled human plasma, and could be prone to aggregation. (*Neurology*, 59(Suppl. 6): S28-S32, at S28; entered into the record on May 11, 2010; copy attached.) The review also includes a table of the then-available polyclonal IgG preparations on the U.S. pharmaceutical market. (*Id.* at S29.)

Independent Claim 29 and Its Dependent Claims

Claim 29 recites a "stable polyclonal IgG preparation, wherein the preparation comprises polyclonal IgG and a stabilizer comprising proline, has a pH of about 4.2 to about 5.4, and does not comprise nicotinamide." The specification discusses "polyclonal IgG preparations" throughout the text, for example, at page 1, line 9, to page 3, line 17, and page 5, lines 14-24. Pages 2 and 3 of the specification, for example, refer to art that addresses the stabilization of polyclonal IgG preparations against formation of dimers and other aggregates, which can cause anaphylactic shock or other reactions, such as fever, nausea, low blood pressure, or hypotension, that be detrimental to pharmaceutical use. (*See* page 1, lines 9-14; page 2, lines 25-32.)

The working examples are also performed using a polyclonal IgG preparation, as described at page 8, line 20, to page 15, for which the starting material is an intermediate from a well-known ethanol fractionation process from pooled plasma samples. (*See* page 8, lines 22-26; *see also* Tankersley at page 160.) The working examples then evaluate the formation of dimers, aggregates, and degradation fragments in the presence of different combinations of pH, temperature, and additives. (*See* pages 9-15.)

Proline as a stabilizer of polyclonal IgG preparations is discussed or exemplified throughout the application, such as at page 4, lines 12-14, pages 10-11, and page 12, Table 1. A pH of about 4.2 to about 5.4 is described, for example, at page 3, lines 28-31, and page 5, lines 26-32, and page 7, complete page. A preparation that "does not comprise nicotinamide" is described, for example, at page 4, lines 9-10.

Claims 30-40 and 45 depend from claim 29. Dependent claims 30, 31, 35, 37, 39, and 45 each further recite, at least in part, particular concentration ranges of IgG in the preparation. The claimed IgG concentration ranges are described, for example, at page 6, lines 14-15, 20-21, and 24. Claims 32 and 37 recite that the "preparation has a pH of about 4.6 to about 5.0." That pH range is described, for example, at page 5, lines 30-32. Claims 33, 35, 37, and 39 recite that the "proline is L-proline" and that the concentration of L-proline in the preparation "is from 0.2 to 0.3 M" or "is from 0.2 to 0.4 M." Page 4, lines 12-13, for example, refers to L-proline, while page 6, line 7, for example, discloses those concentration ranges. Finally, claims 34, 36, 38, and 40 recite that that the preparation "is a liquid preparation that has not been lyophilized and is not lyophilized prior to administration." Page 3, lines 3-5, refers to preparations that are stored in non-lyophilized liquid form. Furthermore, as noted at page 1, lines 16-20, of the application, such a liquid preparation contrasts with one that is lyophilized (i.e. freeze-dried) before storage and later reconstituted prior to administration.

Independent Claim 41 and Its Dependent Claims

Claim 41 is an independent claim that recites a "stable liquid polyclonal IgG preparation, wherein the preparation comprises polyclonal IgG and a stabilizer consisting essentially of proline, has a pH of about 4.2 to about 5.4, and wherein the preparation is not lyophilized prior to administration." The recited "polyclonal IgG preparation" is described above. The claimed proline and pH range are disclosed, for example, at page 4, lines 12-14, pages 10-11, and page 12, Table 1, and at page 3, lines 28-31, and page 5, lines 26-32, and page 7, complete page. A "stabilizer consisting essentially of proline" is exemplified throughout the application as a whole,

for example, in Figures 1-4 and in the examples at pages 8-15. Furthermore, page 3, lines 3-5, for example, refers to liquid preparations that are not lyophilized prior to administration. As noted at page 1, lines 16-20, of the application, such a liquid preparation contrasts with one that is lyophilized and reconstituted prior to administration.

Claims 42-44 depend from claim 41 and recite that the proline is L-proline and that the concentration of the L-proline in the preparation is from 0.2 to 0.4 M or is from 0.2 to 0.3 M. Page 4, lines 12-13, for example, refers to L-proline, while page 6, line 7, for example, discloses those concentration ranges. Claims 42-44 also recite particular IgG concentration ranges, which are disclosed, for instance, at page 6, lines 14-15, 20-21, and 24.

Grounds of Rejection

Whether each of claims 29-45, considered separately, is obvious under 35 U.S.C. § 103(a) over U.S. Patent No. 6,171,586 in view of U.S. Patent Publication No. 2005/0142139A1 ("the '586 patent" and "the '139 publication," respectively).

Argument

Appellants appeal the rejection of claims 29-45 because the rejection is not a *prima facie* case of obviousness. Most fundamentally, the appealed claims recite "polyclonal IgG preparations." Polyclonal IgGs have unique stability problems, which the proteins in the two cited references do not have. The cited references also do not point to the excipients and pH ranges claimed here. Instead, they disclose a large multitude of possible excipients, and teach optimal excipient and pH conditions that are quite distinct from those claimed here. The Office has not sufficiently explained how one would get to the specific preparations claimed here from references that do not address the particular stability problems of polyclonal IgGs and that otherwise point away from the claimed conditions. In addition, the Office Actions do not specifically consider the currently pending claims other than claim 29. Appellants also provided evidence of unexpected results and commercial success commensurate in scope with claims 29-45. But that evidence was only considered in light of claim 29.

Appellants address the Office's obviousness rejection in detail in Section II below, and argue each of claims 29-45 separately.

I. Legal Standards of Obviousness

Several basic factual inquiries must be made to determine whether the claims of a patent application are obvious under 35 U.S.C. § 103. These factual inquiries are set forth in *Graham v. John Deere Co.*, 383 U.S. 1, 17 (1966), and require the Office to:

(1) Determine the scope and content of the prior art;

(2) Ascertain the differences between the prior art and the claims in issue;

(3) Resolve the level of ordinary skill in the pertinent art; and

(4) Evaluate evidence of secondary considerations.

The obviousness or non-obviousness of the claimed invention is then evaluated in view of the results of these inquiries. *Graham*, 383 U.S. at 17-18; *see also KSR Int'l Co. v. Teleflex, Inc.*, 127 S. Ct. 1727, 1734 (2007). Moreover, "rejections on obviousness cannot be sustained with mere conclusory statements; instead, there must be some articulated reasoning with some rational underpinning to support the legal conclusion of obviousness." *See KSR*, 127 S. Ct. at 1741 (quoting *In re Kahn*, 441 F.3d 977, 988 (Fed. Cir. 2006)); M.P.E.P. § 2142. The Office has emphasized in its *2010 Examination Guidelines Update: Developments in the Obviousness Inquiry After* KSR v. Teleflex that examiners must continue to provide a reasoned explanation as to why the invention as claimed would have been obvious to a person of ordinary skill in the art at the time of the invention. *KSR*, at 1741; 75 Fed. Reg. 53643-60 at 53645, second column.

Hindsight reasoning is also improper. *See KSR*, at 1742-43. Instead, "[t]o reach a proper determination under 35 U.S.C. § 103, one must step backward in time and into the shoes worn by the hypothetical 'person of ordinary skill in the art' when the invention was unknown and just before it was made. In view of all factual information, the Office must then make a determination whether the claimed invention 'as a whole' would have been obvious at the time to that person." M.P.E.P. § 2142.

Furthermore, if a *prima facie* case of obviousness is to be based upon a combination of prior elements, the Office must provide sufficient reasoning or evidence to show that the combination yields a predictable outcome or that there is a reasonable expectation of success. M.P.E.P. §§ 2143 and 2143.02. Predictability, according to the

Federal Circuit, means not only that there is an expectation that the prior art elements can be combined or modified, but also that there is an expectation that the resulting combination or modification will work for its intended purpose. *DePuy Spine, Inc. v. Medtronic Sofamor Danek, Inc.*, 567 F.3d 1314 (Fed. Cir. 2009); *see also 2010 Examination Guidelines Update*, 75 Fed. Reg. at 53649, first column. And even where a new combination is considered "obvious to try," the Office must establish (1) that there are only a finite number of possible solutions (2) with predictable outcomes and (3) that there is a reasonable expectation of success in pursuing those possible solutions. *KSR*, at 1742; M.P.E.P. § 2143(E).

Finally, even if a *prima facie* case of obviousness is established, it may be rebutted by evidence of secondary considerations such as unexpected results and commercial success commensurate in scope with the patent claim. M.P.E.P. § 2142.

II. The '586 Patent and '139 Publication Do Not Render Any of the Appealed Claims 29-45 Obvious

A. Independent Claim 29

Claim 29 recites a "stable polyclonal IgG preparation, wherein the preparation comprises polyclonal IgG and a stabilizer comprising proline, has a pH of about 4.2 to about 5.4, and does not comprise nicotinamide."

1. The '586 Patent and '139 Publication Do Not Relate to Polyclonal IgG Preparations

The instant obviousness rejection suffers from a critical flaw in that neither the '586 patent nor the '139 publication[1] provides adequate guidance about how to formulate and stabilize a "polyclonal IgG preparation." The claimed "polyclonal IgG preparations" have unique stability challenges. Not only do these references fail to address those stability challenges, the references do not even discuss polyclonal IgG preparations.

Polyclonal IgG preparations are generally derived from pools of blood plasma from up to thousands of different individual donors. (*See* Tankersley, at 160-163, entered into the record on June 2, 2011; copy attached.) As a 1994 review by Tankersley explains, by that time, the international regulatory agencies required that a polyclonal IgG preparation for therapeutic use be derived from a large number of different individual donors, for example, 1000 or more. (*Id.* at 161.) For that reason, a polyclonal IgG preparation generally comprises a vast array of different IgG molecules with different amino acid sequences, different antigen specificities, and other different properties. In sharp contrast, most other therapeutic protein preparations contain only one, specific protein species, generally in highly pure form, and which is usually recombinantly produced. (*See Id.* at 161-163*; see also* specification at page 2, lines 24-26, page 10, lines 14-21, and Tables 1 and 2.)

Polyclonal IgGs have characteristic stability problems because they are mixtures of many different IgG species. In particular, polyclonal IgG preparations are prone to a

[1] The Office has applied the '139 publication as prior art under 35 U.S.C. § 102(e). The application corresponding to that publication was filed March 19, 2004, and claims priority to a provisional U.S. patent application filed March 21, 2003.

specific type of dimerization, called an idiotype –anti-idiotype interaction, that occurs because different antibody species from different donors within a preparation recognize and bind to each other. (Tankersley at 161-162.) This dimerization can result in adverse events in patients, such as hypotension, and is therefore important to control. (*See* Tankersley, at page 160; specification at page 2, lines 25-32.) The '586 patent and '139 publication do not provide any guidance about controlling idiotype –anti-idiotype dimerization because they concern purified, recombinant proteins that do not suffer from this problem.

Polyclonal IgG preparations are also prone to characteristic aggregation, fragmentation, and oxidation. (*See* specification at pages 9-11, Tables 1 and 2; *see also* Cramer et al., *Vox Sang*. 96: 219-225 (2009) at paragraph bridging pages 219-220, entered into the record on May 11, 2010, copy attached.) Aggregation, for example, can cause dangerous adverse events such as anaphylactic shock. (Tankersley at 160-161; Lemm at S28, entered into the record on May 11, 2010; copy attached; Specification at page 1, lines 9-14.)

Hence, a researcher wishing to make a "stable polyclonal IgG preparation" would have been interested in balancing all of these stability problems together: idiotype–anti-idiotype dimerization, aggregation, fragmentation, and oxidation. (*See, e.g.,* specification at pages 9-11 and Tables 1-2.) The '586 patent and '139 publication do not provide guidance about how to control all of these stability problems because they concern proteins that do not suffer from all of those problems. Accordingly, a person of ordinary skill in the art could not have predicted from either of the cited publications

whether the excipients and pH conditions they describe could effectively stabilize a polyclonal IgG preparation.

In particular, the '586 patent relates to specific recombinant humanized monoclonal antibodies. (See the '586 patent at cols. 24-46 and Figures 1-28.) In other words, this patent refers to preparations comprising a single, purified, recombinantly produced protein, not a complex mixture of different IgG protein species from different plasma donors. Recombinant protein and monoclonal antibody preparations do not have the stability problems associated with the claimed polyclonal IgG preparations because they comprise only one single, purified protein species. Recombinant proteins and monoclonal antibodies cannot suffer from idiotype–anti-idiotype dimerization, for example. Thus, this patent does not provide any guidance on how to solve this dimerization problem.

Furthermore, the preparations of claim 29 require proline, and the Office has acknowledged that the '586 patent "does not teach the use of proline." (Office Action of May 19, 2010, at page 8, last two lines.)

The Office seems to cite the '586 patent because it allegedly discloses "polyclonal antibody" preparations. (Office Action of June 7, 2011, at page 3, second paragraph under section 6.) But Appellants do not claim "polyclonal antibody preparations." Appellants claim "polyclonal IgG preparations," which are distinct from the polyclonal antibodies mentioned in the '586 patent.

The reference to "polyclonal antibodies" in the '586 patent is not relevant to the invention of claim 29. The polyclonal antibodies described at column 11 of the '586 patent are obtained from host animals and directed against a "relevant antigen," in other

words, against a specific antigen target. ('586 patent, col. 11, at line 33.) And they may be derived from only one host animal. ('586 patent, col. 11, at lines 30-58.) Such target-specific antibodies do not develop the idiotype–anti-idiotype dimerization that causes stability and adverse event problems for polyclonal IgG preparations. Moreover, even if polyclonal antibodies were relevant here, the '586 patent does not provide any data or working examples showing preparations of polyclonal antibodies. All of the experimental data the '586 patent are based on a recombinant protein. (*See, e.g.,* the '586 patent at col. 24, lines 29-38, and col. 27, lines 57-58.) The brief mention of "polyclonal antibodies" in the '586 patent would not have provided any guidance to a person of ordinary skill in the art who wished to stabilize a polyclonal IgG preparation.

Thus, the Office's remarks do not pertain to the pending claims. And, even if the '586 patent's reference to "polyclonal antibodies" had any relevance to the appealed claims, the patent does not provide any guidance as to how proline affects their stability. Thus, the '586 patent is simply not relevant to claim 29.

The Office cites the '139 publication for a teaching of proline. But that publication also deals with a single, recombinantly-made protein species in pure form. Such a protein cannot suffer from idiotype–anti-idiotype dimerization.[2] Thus, the '139 publication also provides no guidance as to how to solve the problems encountered in making a stable polyclonal IgG preparation as recited in claim 29.

[2] In fact, the protein referred to in the '139 publication cannot suffer from idiotype–anti-idiotype dimerization also because it is not an immunoglobulin and does not comprise the variable domain regions of an immunoglobulin that may cause this dimerization. Instead, that protein is a chimeric molecule in which the Fc, hinge, and first domain of the Fab region of an immunoglobulin are fused to another protein called CD4.

As a whole, because these two references do not consider polyclonal IgG preparations, which have stability issues that pure protein species do not have, they do not provide guidance as to how to stabilize a polyclonal IgG preparation.

2. The Office Actions Do Not Set Forth a *Prima Facie* Case

The Office Actions state that "[o]ne of ordinary skill in the art at the time the invention was made would have been motivated to [combine the '586 patent and '139 publication] because the addition of proline improves stability of protein upon storage and delivery by reducing aggregation." (Office Action of June 7, 2011, at page 5; Office Action of May 19, 2010, at page 9.) The Office provides no support whatsoever for that statement, either in the cited publications or elsewhere in the scientific literature. The Office Actions then state that "there would have been a reasonable expectation of success in producing the claimed invention." (*Id.*) Again, no supportive reasoning is provided.

These bare statements are conclusory and do not provide the level of reasoning that is required under the Office's post-KSR guidelines to create a *prima facie* case. *See USPTO Examination Guidelines Update: Developments in the Obviousness Inquiry after* KSR v. Teleflex, 75 Fed. Reg. 53643-72 (Sept. 1, 2010), and M.P.E.P. § 2141. As the Office has recently pointed out, "[i]t remains Office policy that appropriate factual findings are required in order to apply the enumerated rationales [including the "TSM" test] properly. If a rejection has been made that omits one of the required factual findings, and in response to the rejection a practitioner or inventor points out the omission, Office personnel must either withdraw the rejection, or repeat the rejection including all required factual findings." 75 Fed. Reg. 53643 at 53645, col. 1 (Sept. 1,

2010). "This requirement for explanation remains even in situations in which Office personnel may properly rely on intangible realities such as common sense and ordinary ingenuity." *Id.* at 53645, col. 2.

In response to Appellants' prior arguments that the two references do not relate to "polyclonal IgG preparations," the Office stated that: ". . . the '586 patent clearly defines the polyclonal antibody included in the "antibody" (note col. 7) and the '139 publication teaches use of proline. One cannot show nonobviousness by attacking references individually . . ." (Office Action of June 7, 2011, at 6, first paragraph.) Those remarks do not address the current claim language.

In summary, the Office does not consider that Appellants do not claim "polyclonal antibodies." Appellants instead claim "polyclonal IgG preparations," which are different. Polyclonal IgG preparations have unique stability problems that the proteins discussed in the cited references do not have. The Office Actions do not adequately explain how, from these references, one could predict which stabilizers could solve the problems unique to polyclonal IgG preparations. For these reasons alone, the Office has not set forth a *prima facie* case of obviousness against claim 29.

3. Even If the '586 Patent and '139 Publication Pertained to Polyclonal IgG Preparations, They Do Not Suggest a Stabilizer Comprising Proline at a pH Within the Range Recited in Claim 29

Furthermore, if, for the sake of argument, the '139 publication is considered to apply to polyclonal IgG preparations, its teachings as a whole point away from aspects of the invention of claim 29. In particular, the '139 publication states that its formulation may comprise an amino acid stabilizer that could be selected from alanine, glycine, proline, glycylglycine, leucine, isoleucine, creatinine, arginine, and lysine. (*See*

paragraphs [0032], [0039], and [0137]-[0138].) However, the working examples of the '139 publication mostly use glycine as an amino acid stabilizer. (*See* Tables 2-4 and 7-9; *and see* paragraph [0151].) In fact, the publication explicitly states that glycine is preferred. (*See* paragraph [0039].) The working examples section states that the optimal formulation comprised histidine, glycine, the sugar trehalose, and the surfactant Tween® 80 at pH 6, based on lead formulations comprising glycine. (Paragraph [0151]; *see also* paragraph [0126].) Furthermore, the publication as a whole teaches a higher pH range than is claimed here. (*See* paragraphs [0073], [0074], and [0126].) Thus, even if the '139 publication is viewed as relevant to claim 29 because it mentions proline, the publication as a whole teaches that its optimal formulations use glycine and are at pH 6, which is well above the pH range recited in claim 29 (about 4.2 to about 5.4).

In addition, the '586 patent does not mention proline. In fact, it fails to even mention amino acids as possible stabilizers or formulation excipients. Its proteins are formulated with molecules chosen from surfactants and polyalcohols (also called polyols) at a pH range of about 4.5 to about 6.0. (*See* col. 2, lines 35-40; col. 6, lines 38-60; col. 22, lines 31-59; col. 23, lines 1-14.) The patent teaches that these polyols include compounds such as fructose, mannose, maltose, lactose, arabinose, xylose, ribose, rhamnose, galactose, glucose, sucrose, trehalose, sorbose, melezitose, raffinose, mannitol, xylitol, erythritol, threitol, sorbitol, glycerol, and L-gluconate, while surfactants include compounds such as "polysorbates (e.g. polysorbates 20, 80, etc.) or poloxamers (e.g. poloxamer 188)." (Col. 6, lines 38-60; col. 22, lines 31-59.) The patent's exemplary formulations use combinations of the polyols D-mannitol and

trehalose and the surfactant Tween® 20 at pH 5-6. (*See* Tables 2 and 5 at cols. 28 and 29.)

Assuming only for the sake of argument that a person of ordinary skill in the art would find these disclosures predictive of what would happen in formulating a polyclonal IgG preparation, that person, knowing nothing of Appellants' invention, would have had to arrive at the formulation of claim 29 by picking out proline as a potential stabilizer from the vast array of compounds described in these two references. And in doing so, that person also would have had to ignore or act contrary to the optimal formulations presented in each reference. This demonstrates that the Office could only have selected the two cited references from improper hindsight.

At the very best, the two cited references show that there was a large number of possible compounds one could try out as stabilizing ingredients for a protein formulation, and that proline in the absence of nicotinamide was merely one possibility out of many. Thus, there was no "finite number of [previously] identified, predictable solutions" in the art for a person of ordinary skill knowing nothing of the invention of claim 29 to try. And these references do not single out proline from any of the other disclosed possible stabilizers. In fact, taken as a whole, they indicate that glycine, trehalose, and the Tween® 20 and 80 surfactants work best for the purified, recombinant proteins that they each concern.

Moreover, Appellants' invention should also be considered in light of the then-available commercial polyclonal IgG preparations. When the instant application was filed, these preparations were generally stabilized with combinations of specific sugars, sugar alcohols or polyols, surfactants, and albumin protein. (*See, e.g.,* European

Patent No. 1 084 147B1, table at page 13; Buckley et al., *N. Engl. J. Med.* 325(2): 110-117 (1991), at page 111, Table 1; each entered into the record on October 30, 2009, copies attached.) As can be seen from EP 1 084 147B1 and Buckley et al., many prior commercial formulations were lyophilized and were stabilized by:

- maltose, glycine, and albumin (Gammagard®)

- sucrose and albumin (Gammar IV®)

- sucrose and PEG (Iveegam®)

- sucrose (Sandoglobulin®)

- D-mannitol, albumin, and PEG (Venoglobulin®), and

- maltose and albumin (Gammonativ®).

Available liquid formulations were stabilized, for example, by:

- maltose and glucose (Octagam®), and

- maltose (Gamimune®).

These formulations certainly should have been known to a person of ordinary skill in this art and would have suggested continuing to use mixtures of sugars, sugar alcohols, PEG, and albumin. The preparation of claim 29 represents a significant departure from these prior products, which is further evidence of its patentability.

4. The Claimed Invention Shows Unexpected Results and Commercial Success

Even if, purely for the sake of argument, one assumes that a *prima facie* case of obviousness has been established, polyclonal IgG preparations of claim 29 show an unexpectedly high stability and a low level of toxic side effects. Appellants have provided information regarding two commercial products encompassed by claim 29.

(Reply to Office Action under 37 C.F.R. § 1.114, Information Disclosure Statement, and PTO Form SB/08 of September 17, 2010.)

Privigen® is a liquid polyclonal IgG preparation comprising about 10% polyclonal IgG formulated with 210 to 290 mM L-Proline at pH of about 4.8, and which is not lyophilized before administration. Appellants have submitted the U.S. Food and Drug Administration approved package insert, an article by M. Cramer et al., and an *International Blood/Plasma News* excerpt from April, 2010. These materials were entered into the record on June 2, 2011, and copies are also attached.

The *International Blood/Plasma News* excerpt and the Cramer article both describe the exceptional stability of Privigen® compared to other available polyclonal IgG preparations. The *International Blood/Plasma News* excerpt states that "U.S. FDA has approved a supplemental Biologics License Application (sBLA) that extends the shelf life of [CSL Behring's] *Privigen* 10% liquid intravenous immunoglobulin product from 24 months to 36 months. This approval makes *Privigen* the first liquid IVIG in the U.S. that can be stored at room temperature (up to 25˚C [77˚F]) throughout its entire 36-month shelf life." Thus, Privigen® is the first liquid polyclonal IgG preparation, not lyophilized prior to administration, that is stable at room temperature for three years.

The Cramer article, published in 2009, contrasts the stability of Privigen® to that of other liquid polyclonal IgG preparations available at that time. Cramer et al. state that the "liquid IVIG formulations have limited shelf-lives. Long-term storage of liquid IVIG formulations has therefore required the use of refrigerated conditions until now." (Page 219, column 1; Discussion, first paragraph.) The Cramer article notes that polyclonal IgG preparations at a lower IgG concentration of 5% "may be stored at room

temperature for a period of up to 1 year" and that "[t]he optimal storage temperature for currently available 10% IVIG solutions is 5 ℃, for a maximal storage time of 36 months [3 years]. These solutions are stable at room temperature for only a few months." (Discussion section, first column, page 225, emphasis added.) Thus, Privigen®, despite its higher concentration of polyclonal IgG than competing liquid polyclonal IgG preparations, is about three-times more stable. (*See Id.*) And again, Privigen®'s three years at room temperature shelf life was approved by the U.S. Food and Drug Administration as the longest shelf life among approved liquid polyclonal IgG preparations. (*See* the *International Blood/Plasma News* excerpt.)

The instant claims also cover another commercial polyclonal IgG preparation called Hizentra™. Hizentra™ was approved by the U.S. Food and Drug Administration in February, 2010. (Hizentra™ FDA package insert, entered into the record on June 2, 2011, copy attached.) It is a 20% liquid polyclonal IgG preparation comprising 210-290 mM proline and 10-30 mg/liter polysorbate 80, trace sodium, with a pH of 4.6 to 5.2, and is not lyophilized prior to administration. Hizentra™ is the most concentrated liquid, non-lyophilized polyclonal IgG preparation available on the U.S. market. In August, 2010, the FDA extended Hizentra™'s shelf life from 18 to 24 months without refrigeration. (August 18, 2010, CSL Behring press release; entered into the record on June 2, 2011, copy attached.) Subsequently, in February, 2011, the FDA further extended the shelf life to 30 months at room temperature.

Given that commercial polyclonal IgG preparations are all obtained from pooled human plasma, the commercial success of Privigen® and Hizentra™ must be largely due to their unique formulations, which allow for a long shelf-life at room temperatures,

lack of a need for lyophilization and reconstitution by hospital staff, and a higher protein concentration. Products with a long shelf life, that can be stored at room temperature and with no need for reconstitution are easier for hospital staff to store and use, and result in less wastage. (*See, e.g.,* Cramer at page 219 under "Introduction"; Lemm, at S30, first column under "liquid v. lyophilized IVIg preparations.") The higher protein concentrations mean that intravenous transfusion volumes can be reduced, which is safer and easier for patients, and simpler dosing methods can be employed, such as subcutaneous injection in place of the standard intravenous infusion. (*See* Lemm at S30 under "IVIg concentration.")

Furthermore, protein formulation is inherently unpredictable. Trial and error experimentation is generally required to determine whether a particular excipient can adequately stabilize a polyclonal IgG preparation against formation of dimers, aggregates, fragments, and oxidation products. (*See, e.g.,* Declaration of R. Bolli under 37 C.F.R. § 1.132, submitted February 9, 2009, at paragraphs 11-13, copy attached.) Due to the general unpredictability of successfully minimizing the formation of dimers, aggregates, fragments, and oxidation products in a polyclonal IgG, one of ordinary skill in the art would not have expected that, by using proline in the absence of nicotinamide, one could achieve such high stability at room temperature even with higher protein concentrations than ever used before.

The Office appears to have acknowledged that the stabilities of Privigen® and Hizentra™ are unexpected in view of the art. (*See* Office Action of June 7, 2011, at page 6, third paragraph.) However, the Office states that "the scope of the claimed invention is not commensurate with the references." (*Id.* at page 6, fourth and fifth

paragraphs.) In particular, the Office comments that claim 29 is open to other stabilizers aside from nicotinamide. (*See Id.*)

The Office did not consider, however, that Hizentra™ contains such an additional stabilizer, Tween® 80. And a comparison of Hizentra™ to Privigen® demonstrates that when one adds that further Tween® 80 stabilizer to the proline, the polyclonal IgG preparation is even more stable than when using proline alone. (*See* Office Action of June 7, 2011, at page 6, final paragraph.) For instance, the protein concentration may be doubled, but the shelf-life remains relatively high. Thus the submitted documents concerning Privigen® and Hizentra™ are clearly commensurate in scope with claim 29. (Furthermore, as discussed below, Privigen® and/or Hizentra™ are even more specifically encompassed within claims 35-45.)

For all of the reasons above, Appellants urge the Board to overturn the rejection of claim 29.

B. Claim 30

Claim 30 depends from claim 29 and further recites that "the concentration of IgG in the preparation is 8-12% w/v." All of Appellants' remarks with respect to claim 29 also apply to claim 30. Thus, Section II(A) of this Argument is incorporated herein.

Furthermore, the Office contends that the '139 publication refers to protein concentrations from 100-162 mg/ml (10-16.2% w/v). (June 7, 2011, Office Action at 4, fourth paragraph.)[3] But that disclosure is irrelevant to claim 30 because the cited publication refers to a purified, recombinant protein and not to a polyclonal IgG

[3] Appellants also note that the Office's remarks actually refer to "claims 10-13," which are no longer pending, rather than to claim 30.

preparation. Hence, the cited publication does not address the stability concerns related to the claimed preparation. Thus, the Office has not made a *prima facie* case against claim 30 for these reasons, as well as for the reasons provided in Section II(A).

Moreover, the evidence of unexpected results and commercial success applies also to claim 30, in that the concentration of IgG in Privigen® falls within the range of claim 30, while that of Hizentra™ is even higher than the range of claim 30. Both of those preparations also comprise polyclonal IgG and a stabilizer comprising proline, have a pH of about 4.2 to about 5.4, and do not comprise nicotinamide, as recited in claim 29.

For all of the reasons in Section II(A) and above, Appellants urge the Board to overturn the rejection of claim 30.

C. Claim 31

Claim 31 depends from claim 30 and recites that "the concentration of IgG in the preparation is 10% w/v." All of Appellants' remarks with respect to claims 29 and 30 also apply to claim 31. Thus, Sections II(A) and (B) of this Argument are incorporated herein.

Furthermore, while the Office contends that the '139 publication refers to protein concentrations from 100-162 mg/ml (10-16.2% w/v), that disclosure is irrelevant to claim 31 for the same reasons it is irrelevant to claim 30. (June 7, 2011, Office Action at 4, fourth paragraph.) The cited publication refers to a purified, recombinant protein and not to a polyclonal IgG preparation as claimed here, and so, does not address the special stability issues of a polyclonal IgG preparation. Hence, for these reasons, as

well as for all of the reasons in Sections II(A) and (B), the Office has not made a *prima facie* case against claim 31.

Appellants further note that the evidence of unexpected results and commercial success applies also to claim 31, in that the concentration of IgG in Privigen® is 10% w/v, as recited in claim 31, while that of Hizentra™ is even higher than the range of claim 31, while both preparations meet all of the requirements of claims 29 and 30 as well.

For all of the reasons in Sections II(A) and (B) and above, Appellants urge the Board to overturn the rejection of claim 31.

D. Claim 32

Claim 32 depends from claim 29 and recites that the "preparation has a pH of about 4.6 to about 5.0." All of Appellants' remarks with respect to claim 29 also apply to claim 32. Thus, Section II(A) of this Argument is incorporated herein.

In addition, if, for the sake of argument, the combination of the '586 patent and the '139 publication relates to a polyclonal IgG preparation, that combination does not suggest the specific pH range of claim 32. Instead, the two documents have conflicting teachings with regard to pH, the '139 publication suggesting an optimal pH of 6.0, and the '586 patent teaching a lower pH range.

In addition, the June 7, 2011, and May 19, 2010, Office Actions do not address claim 32 or its recited pH range. For that reason alone, as well as for the reasons above and provided in Section II(A), the Office has not made a *prima facie* case against claim 32.

Moreover, the evidence of unexpected results and commercial success applies also to claim 32, in that the pH range of both Privigen® and Hizentra™ falls within that of claim 32, while both products also meet the limitations of claim 29.

For all of the reasons in Section II(A) and above, Appellants urge the Board to overturn the rejection of claim 32.

E. Claim 33

Claim 33 depends from claim 29 and recites that the "proline is L-proline, and the concentration of L-proline in the preparation is from 0.2 to 0.3 M." All of Appellants' remarks with respect to claim 29 also apply to claim 33. Thus, Section II(A) of this Argument is incorporated herein.

In addition, if, for the sake of argument, the combination of the '586 patent and the '139 publication relates to a polyclonal IgG preparation, that combination does not suggest L-proline at the specific concentration range of claim 33. Instead, the '586 patent does not mention a stabilizer comprising proline, or any other amino acid, and the '139 publication mentions a concentration of "between about 25-150 mM" and prefers glycine at about 50 mM, which is a lower range than claimed in claim 33. ('139 publication at paragraphs [0013] and [0032], for example.) In addition, the June 7, 2011, Office Action does not specifically address claim 33, although it refers to concentrations allegedly recited in two claims that were previously cancelled. (June 7, 2011, Office Action at page 4, third paragraph, referring to claims 7 and 8.) For these reasons, and the reasons provided in Section II(A), the Office has not made a *prima facie* case of obviousness against claim 33.

Appellants further note that the evidence of unexpected results and commercial success applies also to claim 33, in that both Privigen® and Hizentra™ contain 210-290 mM L-proline, which falls within the claimed range, as well as meet the limitations of claim 29.

For all of the reasons in Section II(A) and above, Appellants urge the Board to overturn the rejection of claim 33.

F. Claim 34

Claim 34 depends from claim 29 and recites that that the preparation "is a liquid preparation that has not been lyophilized and is not lyophilized prior to administration." All of Appellants' remarks with respect to claim 29 also apply to claim 34. Thus, Section II(A) of this Argument is incorporated herein.

Moreover, if, for the sake of argument, the combination of the '586 patent and the '139 publication relates to a polyclonal IgG preparation, that combination does not suggest a liquid preparation as claimed with the other features of claim 34. For example, the '139 publication relates to formulations of a recombinant protein that comprise a "lyoprotectant," and therefore, are intended to be lyophilized, while the '586 patent does not disclose a stabilizer comprising any amino acid, let alone one comprising proline. ('139 publication at paragraph [0010], for example.)

Appellants also note that the assertions in the June 7, 2011, Office Action do not take into account Appellants' amendment to claim 34 of September 17, 2010. Specifically, the Office asserted that the phrase "wherein the preparation is a liquid preparation that has not been subject to lyophilization" could encompass a preparation that is lyophilized and reconstituted at some future time. (*See* June 7, 2011, Office

Action at page 4, second to last paragraph; *and cf.* page 2, paragraph 1, entering Appellants' September 17, 2010, amendments.) But the Office failed to consider that claim 34 currently recites, not only that the preparation is "liquid" and "has not been subject to lyophilization," but also that the preparation "is not lyophilized prior to administration." Hence, for all of the reasons above and in Section II(A), the Office has not made a *prima facie* case of obviousness against claim 34.

Appellants further note that the evidence of unexpected results and commercial success applies also to claim 34, in that both Privigen® and Hizentra™ are liquid preparations that are not lyophilized prior to administration according to claim 34. Yet each composition retains an exceptional stability and shelf-life at room temperature.

For all of the reasons in Section II(A) and above, Appellants urge the Board to overturn the rejection of claim 34.

G. Claim 35

Claim 35 depends from claim 29 and recites that the "proline is L-proline and the concentration of the L-proline in the preparation is from 0.2 to 0.4 M, and wherein the concentration of IgG in the preparation is 6-15% w/v." All of Appellants' remarks with respect to claim 29 also apply to claim 35. Thus, Section II(A) of this Argument is incorporated herein.

In addition, the Office has not set forth any reasoning as to why the particular set of conditions of claim 35 should be found obvious over the cited references. For example, if, for the sake of argument, the combination of the '586 patent and the '139 publication relates to a polyclonal IgG preparation, that combination does not suggest the specific L-proline concentration range of claim 35 and pH range of claim 29 coupled

with a protein concentration range of 6-15% w/v. Instead, the '586 patent does not mention a stabilizer comprising proline ,or any other amino acid, and the '139 publication mentions an amino acid concentration of "between about 25-150 mM" and prefers glycine at about 50 mM, which is a lower range than claimed in claim 33, and at pH 6, which is above the range of claim 29. ('139 publication at paragraphs [0013], [0032], and [0151], for example.) And, while the Office contends that the '139 publication refers to protein concentrations from 100-162 mg/ml (10-16.2% w/v), that disclosure is irrelevant to claim 35. (June 7, 2011, Office Action at 4, fourth paragraph.) The '139 publication refers to a purified, recombinant protein and not to a polyclonal IgG preparation as claimed here, and so, does not address the special stability issues of a polyclonal IgG preparation.

The Office has not provided any reason why a person of ordinary skill in the art knowing nothing of the invention of claim 35 would have picked the particular conditions of that claim. In fact, the Office did not specifically address claim 35 and its combination of features in either the May 19, 2010, or the June 7, 2011, Office Action.

Hence, for these reasons, as well as for all of the reasons in Section II(A), the Office has not made a *prima facie* case of obviousness against claim 35.

Appellants further note that the evidence of unexpected results and commercial success applies also to claim 35, because (1) the concentration of IgG in Privigen® falls within that of claim 35, while that of Hizentra™ is even higher than the range of claim 35, (2) the concentration of L-proline in each of those preparations is 210-290 mM, which falls within the claimed range, and (3) the pH range of each product falls within that of claim 29.

For all of the reasons in Section II(A) and above, Appellants urge the Board to overturn the rejection of claim 35.

H. Claim 36

Claim 36 depends from claim 35, which, in turn, depends from claim 29. Claim 36 recites that the preparation of claim 35 "is a liquid preparation that has not been lyophilized and is not lyophilized prior to administration." All of Appellants' remarks with respect to claims 29 and 35 also apply to claim 36. Thus, Sections II(A) and (G) of this Argument are incorporated herein.

Moreover, the Office has not set forth any reasoning as to why the particular set of conditions of claim 36 should be found obvious over the cited references. For example, if, for the sake of argument, the combination of the '586 patent and the '139 publication relates to a polyclonal IgG preparation, that combination does not suggest the specific L-proline concentration range of claim 35 coupled with a protein concentration of 6-15% w/v, and a pH of about 4.2 to about 5.4, wherein the preparation does not comprise nicotinamide. Instead, the '586 patent does not even mention a stabilizer comprising proline, or any other amino acid, and the '139 publication mentions an amino acid concentration of "between about 25-150 mM" and prefers glycine at about 50 mM, which is a lower range than claimed in claim 35, and a pH 6, which is above the range of claim 29. ('139 publication at paragraphs [0013], [0032], and [0151], for example.) And, while the Office contends that the '139 publication refers to protein concentrations from 100-162 mg/ml (10-16.2% w/v), that disclosure is irrelevant to claim 36. (June 7, 2011, Office Action at 4, fourth paragraph.) The '139 publication refers to

a purified, recombinant protein and not to a polyclonal IgG preparation as claimed here, and so, does not address the special stability issues of a polyclonal IgG preparation.

Yet further, this combination of references does not suggest a preparation comprising the specifically-claimed features of claims 29 and 35 including stabilizer ingredients, pH range, L-proline concentration range, and protein concentration range, and that is also in liquid form and not lyophilized prior to administration. Instead, the '139 publication relates to formulations of a recombinant protein that comprise a "lyoprotectant," and therefore, are intended to be lyophilized, while the '586 patent does not disclose a stabilizer comprising any amino acid, let alone one comprising proline. ('139 publication at paragraph [0010], for example.) The Office has not provided any reason why a person of ordinary skill in the art knowing nothing of the invention of claim 36 would have picked the particular conditions of that claim.

Appellants also note that the Office's assertions in the June 7, 2011, Office Action do not take into account Appellants' amendment to claim 36 of September 17, 2010. Specifically, the Office asserted that the phrase "wherein the preparation is a liquid preparation that has not been subject to lyophilization" could encompass a preparation that is lyophilized and reconstituted at some future time. (*See* June 7, 2011, Office Action at page 4, second to last paragraph; *and cf.* page 2, paragraph 1, entering Appellants' September 17, 2010, amendments.) But the Office failed to consider that claim 36 currently recites, in addition, that the preparation "is not lyophilized prior to administration."

Hence, for all of the reasons above and in Sections II(A) and (G), the Office has not made a *prima facie* case of obviousness against claim 36.

The evidence of unexpected results and commercial success applies also to claim 36, because (1) the concentration of IgG in Privigen® falls within that of claim 35, while that of Hizentra™ is even higher than the range of claim 35, (2) the concentration of L-proline in each of those preparations is 210-290 mM, which falls within the range of claim 35, (3) the pH range of each product falls within that of claim 29, and (4) each of those preparations is a liquid preparation that is not lyophilized prior to administration, as recited in claim 36.

For all of the reasons in Sections II(A) and (G) and provided above, Appellants urge the Board to overturn the rejection of claim 36.

I. Claim 37

Claim 37 depends from claim 29 and recites that the "preparation has a pH of about 4.6 to about 5.0, the proline is L-proline and the concentration of the L-proline in the preparation is from 0.2 to 0.3 M, and wherein the concentration of polyclonal IgG in the preparation is 8-12% w/v." Claims 30, 32, and 33, also recite the polyclonal IgG concentration range, the pH range, or the L-proline concentration range of claim 37, respectively. Thus, all of Appellants' remarks with respect to claims 29, 30, 32, and 33 also apply to claim 37 and Sections II(A), (B), (D), and (E) of this Argument are incorporated herein.

In addition, the combination of cited references does not suggest a polyclonal IgG preparation with the specific set of pH, L-proline concentration, and protein concentration required by claim 37. And the Office has not provided any reason why a person of ordinary skill in the art knowing nothing of the invention of claim 37 would have picked the particular conditions of that claim.

For example, while the Office contends that the '139 publication refers to protein concentrations from 100-162 mg/ml (10-16.2% w/v), that disclosure is irrelevant to claim 37 because the cited publication refers to a purified, recombinant protein and not to a polyclonal IgG preparation. (June 7, 2011, Office Action at 4, fourth paragraph.) Hence, the cited publication does not address the stability concerns related to the claimed preparation. In addition, the combination of the '586 patent and the '139 publication does not suggest the specific pH range of claim 37. Instead, the two documents have conflicting teachings with regard to pH, the '139 publication suggesting an optimal pH of 6.0, and the '586 patent teaching a lower pH range. And the reference combination does not suggest the specific L-proline concentration range of claim 37. Instead, the '586 patent does not mention a stabilizer comprising proline ,or any other amino acid, while the '139 publication mentions a concentration of "between about 25-150 mM" and prefers glycine at about 50 mM, which is lower than the claimed range, and at pH 6, which is higher than the claimed range. ('139 publication at paragraphs [0013] and [0032], for example.)

The Office also did not specifically address claim 37 and its combination of features in either the May 19, 2010, or the June 7, 2011, Office Action. The June 7, 2011, Office Action, for example, provides no specific remarks regarding this claim.

For all of the reasons above, as well as for the reasons provided in Sections II(A), (B), (D), and (E), the Office has not made a *prima facie* case of obviousness against claim 37.

Moreover, the evidence of unexpected results and commercial success applies also to claim 37, because (1) the concentration of IgG in Privigen® falls within that of

claim 37, while that of Hizentra™ is even higher than the range of claim 37, and (2) the concentration of L-proline in each of those preparations is 210-290 mM, which falls within the claimed range.

For all of the reasons in Sections II(A), (B), (D), and (E), and provided above, Appellants urge the Board to overturn the rejection of claim 37.

J. Claim 38

Claim 38 depends from claim 37, which depends from claim 29. Claim 38 recites that the preparation or claim 37 "is a liquid preparation that has not been lyophilized and is not lyophilized prior to administration." All of Appellants' remarks with respect to claims 29 and 37 also apply to claim 38. Thus, Sections II(A), (B), (D), (E), (F), (H), and (I) of this Argument are incorporated herein.

In particular, the combination of cited references does not suggest a polyclonal IgG preparation with the specific set of pH, L-proline concentration, and protein concentration required by claim 37. For example, while the Office contends that the '139 publication refers to protein concentrations from 100-162 mg/ml (10-16.2% w/v), that disclosure is irrelevant to claim 38 because the cited publication refers to a purified, recombinant protein and not to a polyclonal IgG preparation. (June 7, 2011, Office Action at 4, fourth paragraph.) Hence, the cited publication does not address the stability concerns related to the claimed preparation. In addition, the combination of the '586 patent and the '139 publication does not suggest the specific pH range of claim 37. Instead, the two documents have conflicting teachings with regard to pH, the '139 publication suggesting an optimal pH of 6.0, and the '586 patent teaching a lower pH range. And the reference combination does not suggest the specific L-proline

concentration range of claim 37. Instead, the '586 patent does not mention a stabilizer comprising proline, or any other amino acid, and the '139 publication mentions a concentration of "between about 25-150 mM" and prefers glycine at about 50 mM, which is much lower than the claimed range, and at pH 6, which is higher than the claimed range. ('139 publication at paragraphs [0013] and [0032], for example.)

Furthermore, this combination of references does not suggest a preparation comprising the specifically-claimed features of claim 37 including stabilizer ingredients, pH range, L-proline concentration range, and protein concentration range, and that is also in liquid form and not lyophilized prior to administration, as recited in claim 38. Instead, the '139 publication relates to formulations of a recombinant protein that comprise a "lyoprotectant," and therefore, are intended to be lyophilized, while the '586 patent does not disclose a stabilizer comprising any amino acid, let alone one comprising proline. ('139 publication at paragraph [0010], for example.)

The Office also did not specifically address claim 38 and its combination of features in either the May 19, 2010, or the June 7, 2011, Office Action. For all of the reasons above, as well as for the reasons provided in Sections II(A), (B), (D), (E), (F), (H), and (I), the Office has not made a *prima facie* case of obviousness against claim 38.

The evidence of unexpected results and commercial success applies also to claim 38, because (1) the concentration of IgG in Privigen® falls within that of claim 37, while that of Hizentra™ is even higher than the range of claim 37, (2) the concentration of L-proline in each of those preparations is 210-290 mM, which falls within the range of claim 37, (3) the pH range of each of those commercial preparations falls within that of

claim 37, and (4) each of those preparations is a liquid preparation and is not lyophilized prior to administration, as recited in claim 38.

For all of the reasons in Sections II(A), (B), (D), (E), (F), (H), and (I), and provided above, Appellants urge the Board to overturn the rejection of claim 38.

K. Claim 39

Claim 39 depends from claim 29 and recites that the "proline is L-proline and the concentration of the L-proline in the preparation is from 0.2 to 0.4 M, and wherein the concentration of IgG in the preparation is 15-20% w/v." Claim 35 also recites the same L-proline concentration range as claim 39. Thus, all of Appellants' remarks with respect to claims 29 and 35 also apply to claim 39 and Sections II(A) and (G) of this Argument are incorporated herein.

Moreover, if, for the sake of argument, the combination of the '586 patent and the '139 publication relates to a polyclonal IgG preparation, that combination does not suggest the specific L-proline concentration range of claim 39 coupled with the protein concentration of 15-20% w/v at a pH of about 4.2 to about 5.4. Instead, the '586 patent does not mention a stabilizer comprising proline ,or any other amino acid, and the '139 publication mentions a concentration of "between about 25-150 mM" and prefers glycine at about 50 mM, which is much lower than the claimed range, and at pH 6, which is higher than the claimed range. ('139 publication at paragraphs [0013], [0032], and [0151], for example.) And, while the Office contends that the '139 publication refers to protein concentrations from 100-162 mg/ml (10-16.2% w/v), that disclosure is irrelevant to claim 39. (June 7, 2011, Office Action at 4, fourth paragraph.) The '139 publication refers to a purified, recombinant protein and not to a polyclonal IgG preparation as

claimed here, and so, does not address the special stability issues of a polyclonal IgG preparation.

Yet further, the polyclonal IgG concentration range recited in claim 39 is significantly higher than that of any commercial polyclonal IgG preparation on the U.S. market when this application was filed (e.g., about 5-10% w/v compared to the claimed 15-20% w/v). Neither of the cited references cited suggests that such a highly concentrated polyclonal IgG preparation could be obtained using a stabilizer comprising L-proline from 0.2 to 0.4 M, a pH of about 4.2 to about 5.4, and not comprising nicotinamide. Nor has the Office provided any reasoning to support a contention of obviousness with respect to claim 39. The June 7, 2011, Office Action provides no specific remarks regarding this claim.

Thus, for the reasons above as well as for all of the reasons provided in Sections II(A) and (G), the Office has not provided a *prima facie* case against claim 39.

Appellants further note that the evidence of unexpected results and commercial success applies also to claim 39, because (1) the concentration of IgG in Hizentra™ falls within that of claim 39, (2) the concentration of L-proline in Hizentra™ is 210-290 mM, which falls within the range of claim 39, and (3) the Hizentra™'s pH is within the range of claim 29.

For all of the reasons in Sections II(A) and (G) and provided above, Appellants urge the Board to overturn the rejection of claim 39.

L. Claim 40

Claim 40 depends from claim 39, which in turn, depends from claim 29. Claim 40 recites that the preparation "is a liquid preparation that has not been lyophilized and is

not lyophilized prior to administration." All of Appellants' remarks with respect to claims 29 and 39 also apply to claim 40. Thus, Sections II(A), (G), and (K) of this Argument are incorporated herein.

Moreover, if, for the sake of argument, the combination of the '586 patent and the '139 publication relates to a polyclonal IgG preparation, that combination does not suggest the specific L-proline and protein concentration ranges of claim 39 at a pH of about 4.2 to about 5.4 as recited in claim 29. Instead, the '586 patent does not mention a stabilizer comprising proline ,or any other amino acid, and the '139 publication mentions a concentration of "between about 25-150 mM" and prefers glycine at about 50 mM, which is a lower range than claimed in claim 39, and at pH 6, which is above the range of claim 29. ('139 publication at paragraphs [0013], [0032], and [0151], for example.) And, while the Office contends that the '139 publication refers to protein concentrations from 100-162 mg/ml (10-16.2% w/v), that disclosure is irrelevant to claim 40. (June 7, 2011, Office Action at 4, fourth paragraph.) The '139 publication refers to a purified, recombinant protein and not to a polyclonal IgG preparation as claimed here, and so, does not address the special stability issues of a polyclonal IgG preparation.

In fact, the polyclonal IgG concentration range recited in claim 39 is significantly higher than that of any commercial polyclonal IgG preparation on the U.S. market when this application was filed (e.g., about 10% w/v compared to the claimed 15-20% w/v). Neither of the references cited by the Office suggests that such a highly concentrated polyclonal IgG preparation could be obtained using a stabilizer comprising L-proline from 0.2 to 0.4 M, a pH of about 4.2 to about 5.4, and not comprising nicotinamide.

Even further, this combination of references does not suggest a preparation with such a high polyclonal IgG concentration range, as well as the specifically recited stabilizer and pH requirements, that is also in liquid form and not lyophilized prior to administration. Instead, the '139 publication relates to formulations of a recombinant protein that comprise a "lyoprotectant," and therefore, are intended to be lyophilized, while the '586 patent does not disclose a stabilizer comprising any amino acid, let alone one comprising proline. ('139 publication at paragraph [0010], for example.) The Office has not provided any reason why a person of ordinary skill in the art knowing nothing of the invention of claim 40 would have picked the particular conditions of that claim.

In addition, the Office's assertions in the June 7, 2011, Office Action do not take into account Appellants' amendment to claim 40 of September 17, 2010. Specifically, the Office asserted that the phrase "wherein the preparation is a liquid preparation that has not been subject to lyophilization" could encompass a preparation that is lyophilized and reconstituted at some future time. (*See* June 7, 2011, Office Action at page 4, second to last paragraph; *and cf.* page 2, paragraph 1, entering Appellants' September 17, 2010, amendments.) But the Office failed to consider that claim 40 currently recites, in addition, that the preparation "is not lyophilized prior to administration."

For all of the reasons above as well as for all of the reasons provided in Sections II(A), (G),and (K), the Office has not provided a *prima facie* case against claim 40.

Appellants further note that the evidence of unexpected results and commercial success applies also to claim 40, because (1) the concentration of IgG in Hizentra™ falls within that of claim 39, (2) the concentration of L-proline in Hizentra™ is 210-290 mM, which falls within the range of claim 39, (3) Hizentra™'s pH is within the range of

claim 29, and (4) Hizentra™ is a liquid preparation that is not lyophilized prior to administration.

For all of the reasons in Sections II(A), (G), and (K), and provided above, Appellants urge the Board to overturn the rejection of claim 40.

M. Claim 45

Claim 45 depends from claim 39, which, in turn, depends from claim 29. It recites that "the concentration of IgG in the preparation is 20% w/v." All of Appellants' remarks with respect to claims 29 and 39 also apply to claim 45. Thus, Sections II(A), (G), and (K) of this Argument are incorporated herein.

Moreover, if, for the sake of argument, the combination of the '586 patent and the '139 publication relates to a polyclonal IgG preparation, that combination does not suggest the specific, claimed L-proline concentration range coupled with a protein concentration of 20% w/v at a pH of about 4.2 to about 5.4. Instead, the '586 patent does not mention any stabilizer comprising proline, or any other amino acid, while the '139 publication mentions an amino acid concentration of "between about 25-150 mM" and prefers glycine at about 50 mM, which is much lower range than claimed here, and at pH 6, which is higher than claimed here. ('139 publication at paragraphs [0013], [0032], and [0151], for example.) And, while the Office contends that the '139 publication refers to protein concentrations from 100-162 mg/ml (10-16.2% w/v), that disclosure is irrelevant to claim 40. (June 7, 2011, Office Action at 4, fourth paragraph.) The '139 publication refers to a purified, recombinant protein and not to a polyclonal IgG preparation as claimed here, and so, does not address the special stability issues of a polyclonal IgG preparation.

In addition, the 20% w/v polyclonal IgG concentration recited in claim 45 is at least twice that of any commercial polyclonal IgG preparation on the U.S. market when this application was filed (e.g., about 5-10%). Neither of the references cited by the Office refers to a protein preparation at such a highconcentration, or suggests that such a highly concentrated polyclonal IgG preparation could be obtained using a stabilizer comprising L-proline from 0.2 to 0.4 M, a pH of about 4.2 to about 5.4, and not comprising nicotinamide. Nor has the Office provided any reasoning to support a contention of obviousness with respect to claim 45. The June 7, 2011, Office Action provides no specific remarks regarding this claim.

Thus, for the reasons above, as well as those provided in Sections II(A), (G), and (K), the Office has not provided a *prima facie* case against claim 45.

Appellants further note that the evidence of unexpected results and commercial success applies also to claim 45, because the concentration of IgG in Hizentra™ is 20% w/v, as recited in claim 45, the concentration of L-proline in Hizentra™ is 210-290 mM, which falls within the claimed range, while the pH range of Hizentra™ also falls within the claimed range.

For all of the reasons in Sections II(A), (G), and (K), and provided above, Appellants urge the Board to overturn the rejection of claim 45.

N. Independent Claim 41

Claim 41 is an independent claim that recites a "stable liquid polyclonal IgG preparation, wherein the preparation comprises polyclonal IgG and a stabilizer consisting essentially of proline, has a pH of about 4.2 to about 5.4, and wherein the preparation is not lyophilized prior to administration."

In contrast to independent claim 29, addressed in Section II(A), claim 41 recites that the preparation is "liquid" and "is not lyophilized prior to administration." Thus, the preparation, once made, is not lyophilized and reconstituted. In addition, the "stabilizer consists essentially of proline."

The Office never specifically considered independent claim 41 in either the May 19, 2010, or the June 7, 2011, Office Actions. For that reason alone, the Office has not established a *prima facie* case of obviousness with respect to claim 41.

Nonetheless, because all of Appellants' arguments presented in Section II(A) regarding independent claim 29 also apply to independent claim 41, Section II(A) is incorporated herein.

Furthermore, the '586 and '139 publications do not suggest that a polyclonal IgG preparation could remain stable for its intended use with a stabilizer consisting essentially of proline and while in the liquid form. As Appellants previously outlined in Section II(A)(1), neither publication relates to polyclonal IgG preparations, but to preparations of individual, purified, recombinant proteins. Polyclonal IgG preparations have unique stability problems because they are mixtures of many different immunoglobulin species that may form unwanted dimers as well as higher order aggregates. They may also oxidize and degrade. In contrast, the pure preparations of a single protein species disclosed in the '586 and '139 publications do not face such dimerization problems, for example. Thus, a person of ordinary skill could not determine whether the excipients taught in those two publications could solve the dimerization problem unique to polyclonal IgG preparations.

In addition, neither publication presents any information to predict that a stabilizer consisting essentially of proline at the claimed pH range could adequately stabilize a liquid polyclonal IgG preparation without lyophilization. The '586 patent, in addition to its other defects, does not mention any amino acid as a potential protein stabilizer, let alone proline specifically. Instead, it mentions a vast number of sugars and sugar alcohols and surfactants as putative stabilizers. (*See* Section II(A)(3) above.) And the '139 publication, while it mentions proline, also mentions the amino acids alanine, glycine, glycylglycine, leucine, isoleucine, creatinine, arginine, and lysine. (*See* Tables 2-4 and 7-9; paragraphs [0032], [0039], and [0137]-[0138].) Its working examples mostly use glycine as an amino acid stabilizer. (*See* Tables 2-4 and 7-9.) In fact, the publication explicitly states that glycine is preferred and teaches an optimal formulation of a histidine buffer at pH 6 with glycine, the sugar trehalose, and the surfactant Tween® 80 as stabilizers. (Paragraph [0151]; *see also* paragraph [0126].) Thus, even if the '139 publication is relevant to claim 41 merely because it mentions proline, the publication teaches that its optimal formulations use a mixture of a sugar, surfactant, and glycine, and are also at pH 6, which is well above the pH range recited in claim 41. That disclosure is inconsistent with suggesting a stabilizer consisting essentially of proline.

Appellants simply fail to see how a person of ordinary skill in the art, knowing nothing of the invention of claim 41, could have derived that specific preparation from such unrelated disclosures as the '586 patent and '139 publication. At the very best, those two publications provide a wide range of excipients that one could have tried either alone or in mixtures without providing predictions as to which specific excipients or combinations would adequately solve the unique stability problems of polyclonal

IgGs. This is a far cry from the "finite number of identified, predictable solutions" that the Supreme Court has stated could render a claim obvious as being "obvious to try." *KSR*, at 1742.

The two cited publications should also be considered against the knowledge in the art that the commercially available liquid polyclonal IgG preparations at the relevant time were generally stabilized by maltose and/or glucose sugars. (*See* Section III(A)(3) above.) The invention of claim 41 is a significant departure from such prior products.

The Office did not provide any reason to explain why the two cited references should render claim 41 obvious because the Office Actions never specifically addressed claim 41. Hence, the Office did not provide any *prima facie* case of obviousness against claim 41.

Furthermore, Appellants have provided documentary evidence of both unexpected results and commercial success related to the preparation of claim 41. In particular, the commercial product Privigen® is encompassed by claim 41. Privigen® is a liquid polyclonal IgG preparation comprising about 10% polyclonal IgG formulated with 210 to 290 mM L-Proline at pH of about 4.8, and which is not lyophilized before administration. As noted in Section II(A)(4) above, Appellants have submitted the U.S. Food and Drug Administration approved package insert, a 2009 article by M. Cramer et al., as well as an *International Blood/Plasma News* excerpt from April, 2010, to the Office. The Cramer article and the *International Blood/Plasma News* excerpt explain that Privigen® is the first liquid polyclonal IgG preparation on the U.S. market, not lyophilized prior to administration, that is stable at room temperature for three years.

The Cramer article contrasts the stability of Privigen® with that of other liquid polyclonal IgG preparations available at that time. Cramer et al. state that the "liquid IVIG formulations have limited shelf-lives. Long-term storage of liquid IVIG formulations has therefore required the use of refrigerated conditions until now." (Page 219, column 1; Discussion, first paragraph.) Cramer et al. further state that "[t]hese solutions are stable at room temperature for only a few months." (Discussion section, first column, page 225.) Thus, Privigen® is about three-times more stable than the previously available liquid polyclonal IgG preparations despite the fact that it also has a relatively high polyclonal IgG concentration compared to those other formulations. (*See Id.*; Privigen®'s protein concentration is 10% while other commercial formulations had concentrations ranging from about 5-10%.) And again, Privigen®'s three year, room temperature shelf life was approved by the U.S. Food and Drug Administration as the longest shelf life among the then-available liquid polyclonal IgG preparations. (*See* the *International Blood/Plasma News* excerpt.) All of these benefits attest to the exceptional stability provided by a stabilizer consisting essentially of proline at a pH of about 4.2 to about 5.4.

Furthermore, protein formulation is inherently unpredictable. Trial and error experimentation is generally required to determine whether a particular excipient can adequately stabilize a polyclonal IgG preparation against formation of dimers, aggregates, fragments, and oxidation products. (*See, e.g.,* Declaration of R. Bolli under 37 C.F.R. § 1.132.) Due to the general unpredictability of successfully minimizing the formation of dimers, aggregates, fragments, and oxidation products in a polyclonal IgG, one of ordinary skill in the art would not have expected that, by using a stabilizer

consisting essentially of proline, one could achieve such high stability at room temperature despite retaining a relatively high protein concentration.

The Office never addressed Appellants' evidence of unexpected results and commercial success with respect to claim 41. Instead, the Office's remarks in the June 7, 2011, Office Action refer only to claim 29. (*See, e.g.,* page 6, final paragraph, referring to the term "comprising" being "considered open" and specifically referring to claim 29.)

For all of the reasons above as well as the reasons provided in Section II(A), Appellants urge the Board to overturn the rejection of claim 41.

O. Claim 42

Claim 42 depends from claim 41 and recites that "the proline is L-proline and the concentration of the L-proline in the preparation is from 0.2 to 0.4 M and wherein the concentration of the IgG in the preparation is 6-15% w/v." The additional limitations of claim 42 were also recited in claim 35. Accordingly, the remarks of Section II(N), as well as those made previously in Sections II(A) and (G) also apply to claim 42 and those three sections are incorporated herein.

In addition to failing to provide any specific remarks regarding claim 41, the Office has not set forth any reasoning as to why the particular set of conditions of claim 42 should be found obvious over the cited references. For that reason alone, the Office has not set forth a *prima facie* case with respect to claim 42.

But further, the '586 patent and the '139 publication, added to all of their other deficiencies, do not suggest the specific L-proline concentration range of claim 42 coupled with the pH range of claim 41 and protein concentration range of 6-15% w/v in

a preparation that is liquid and not lyophilized before administration. Instead, the '586 patent does not mention a stabilizer comprising proline, or any other amino acid, and the '139 publication mentions an amino acid concentration of "between about 25-150 mM" and prefers glycine at about 50 mM, which is a lower range than claimed in claim 42, and at pH 6, which is above the range of claim 41. ('139 publication at paragraphs [0013], [0032], and [0151], for example.) And, while the Office contends that the '139 publication refers to protein concentrations from 100-162 mg/ml (10-16.2% w/v), that disclosure is irrelevant to claim 42. (June 7, 2011, Office Action at 4, fourth paragraph.) The '139 publication refers to a purified, recombinant protein and not to a polyclonal IgG preparation as claimed here, and so, does not address the special stability issues of a polyclonal IgG preparation.

For these additional reasons, as well as for all of the reasons in Sections II(A), (G), and (N), the Office has not made a *prima facie* case of obviousness against claim 42.

Appellants further note that the evidence of unexpected results and commercial success applies to claim 42 as well as to claim 41, because (1) the concentration of IgG in Privigen® falls within that of claim 42, (2) the concentration of L-proline in Privigen® is 210-290 mM, which falls within the claimed range, (3) the pH range of Privigen® falls within that of claim 41, and (4) Privigen® is a stable liquid preparation that is not lyophilized prior to administration.

For all of the reasons in Sections II(A), (G), and (N), and given above, Appellants urge the Board to overturn the rejection of claim 42.

P. Claim 43

Claim 43 depends from claim 41 and recites that the "proline is L-proline and the concentration of the L-proline in the preparation is from 0.2 to 0.3 M, and wherein the concentration of IgG in the preparation is 8-12% w/v." The individual limitations of claim 43 were also recited in claims 30 and 33. Hence, the remarks of Section II(N), as well as those made previously in Sections II(A), (B), and (E) also apply to claim 43 and are incorporated herein.

In addition to failing to provide any specific remarks regarding claim 41, the Office has not set forth any reasoning as to why the particular set of conditions of claim 43 should be found obvious over the cited references. For that reason alone, the Office has not set forth a *prima facie* case with respect to claim 43.

Moreover, the '586 patent and the '139 publication, added to all of their other defects, do not suggest the specific L-proline concentration range of claim 43 and pH range of claim 41 coupled with a protein concentration range of 8-12% w/v in a preparation that is liquid and not lyophilized before administration. Instead, the '586 patent does not mention a stabilizer comprising proline, or any other amino acid, and the '139 publication mentions an amino acid concentration of "between about 25-150 mM" and prefers glycine at about 50 mM, which is a lower range than claimed in claim 43, and at pH 6, which is above the range of claim 41. ('139 publication at paragraphs [0013], [0032], and [0151], for example.) And, while the Office contends that the '139 publication refers to protein concentrations from 100-162 mg/ml (10-16.2% w/v), that disclosure is irrelevant to claim 43. (June 7, 2011, Office Action at 4, fourth paragraph.) The '139 publication refers to a purified, recombinant protein and not to a polyclonal IgG

preparation as claimed here, and so, does not address the special stability issues of a polyclonal IgG preparation.

For these additional reasons, as well as for all of the reasons in Sections II(A), (B), (E), and (N), the Office has not made a *prima facie* case of obviousness against claim 43.

Furthermore, the evidence of unexpected results and commercial success applies to claim 43 as well as to claim 41, because (1) the concentration of IgG in Privigen® falls within that of claim 43, (2) the concentration of L-proline in Privigen® is 210-290 mM, which falls within the claimed range, (3) the pH range of Privigen® falls within that of claim 41, and (4) Privigen® is a stable liquid preparation that is not lyophilized prior to administration.

For all of the reasons in Sections II(A), (B), (E), and (N), and given above, Appellants urge the Board to overturn the rejection of claim 43.

Q. Claim 44

Claim 44 depends from claim 41 and recites that the "proline is L-proline and the concentration of the L-proline in the preparation is from 0.2 to 0.4 M, and wherein the concentration of IgG in the preparation is 15-20% w/v." The individual limitations of claim 44 are also recited in claim 39. Hence, all of the remarks of Section II(N), as well as those made previously in Sections II(A) and (K) also apply to claim 44 and are incorporated herein.

In addition to failing to provide any specific remarks regarding claim 41, the Office has not set forth any reasoning as to why the particular set of conditions of claim

44 should be found obvious over the cited references. For that reason alone, the Office has not set forth a *prima facie* case with respect to claim 44.

Moreover, the '586 patent and the '139 publication, added to all of their other deficiencies explained in Sections II(A) and (N), do not suggest the specific L-proline concentration range of claim 44 and pH range of claim 41 coupled with a protein concentration range of 15-20% w/v in a preparation that is liquid and not lyophilized before administration. Instead, the '586 patent does not mention a stabilizer comprising proline, or any other amino acid, and the '139 publication mentions an amino acid concentration of "between about 25-150 mM" and prefers glycine at about 50 mM, which is a lower range than claimed in claim 44, and at pH 6, which is above the range of claim 41. ('139 publication at paragraphs [0013], [0032], and [0151], for example.) And, while the Office contends that the '139 publication refers to protein concentrations from 100-162 mg/ml (10-16.2% w/v), that disclosure is irrelevant to claim 44. (June 7, 2011, Office Action at 4, fourth paragraph.) The '139 publication refers to a purified, recombinant protein and not to a polyclonal IgG preparation as claimed here, and so, does not address the special stability issues of a polyclonal IgG preparation.

For these additional reasons, as well as for all of the reasons in Sections II(A), (K), and (N), the Office has not made a *prima facie* case of obviousness against claim 44.

Furthermore, the evidence of unexpected results and commercial success applies to claim 44 as well as to claim 41. While the concentration of polyclonal IgG in Privigen® is below that of claim 44, the concentration of polyclonal IgG in Hizentra™ is within the range of claim 44. In addition, the concentration of L-proline and pH range in

both Privigen® and Hizentra™ falls within the requirements of claim 44, while both are also stable liquid preparations that are not lyophilized prior to administration. The exceptional stability of both of those commercial formulations indicates that a polyclonal IgG preparation with a stabilizer consisting essentially of L-proline under the conditions of claim 44 should also be sufficiently stable at room temperature to function as a therapeutic.

For all of the reasons in Sections II(A), (K), and (N), and given above, Appellants urge the Board to overturn the rejection of claim 44.

Conclusion

For the reasons given above, elected claims 29-45 are allowable and the rejection of each of claims 29-45 should be reversed.

To the extent any extension of time under 37 C.F.R. § 1.136 is required to obtain entry of this Appeal Brief, such extension is hereby respectfully requested. If there are any fees due under 37 C.F.R. §§ 1.16 or 1.17 that are not enclosed herewith, including any fees required for an extension of time under 37 C.F.R. § 1.136, please charge such fees to Deposit Account No. 06-0916.

Respectfully submitted,

FINNEGAN, HENDERSON, FARABOW,
GARRETT & DUNNER, L.L.P.

Dated: October 3, 2011

By:_____
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Claims Appendix to Appeal Brief Under Rule 41.37(c)(1)(viii)

A listing of the claims on appeal is as follows:

29. A stable polyclonal IgG preparation, wherein the preparation comprises polyclonal IgG and a stabilizer comprising proline, has a pH of about 4.2 to about 5.4, and does not comprise nicotinamide.

30. The preparation of claim 29, wherein the concentration of IgG in the preparation is 8-12% w/v.

31. The preparation of claim 30, wherein the concentration of IgG in the preparation is 10% w/v.

32. The preparation of claim 29, wherein said preparation has a pH of about 4.6 to about 5.0.

33. The preparation of claim 29, wherein said proline is L-proline, and the concentration of L-proline in the preparation is from 0.2 to 0.3 M.

34. The preparation of claim 29, wherein the preparation is a liquid preparation that has not been lyophilized and is not lyophilized prior to administration.

35. The preparation of claim 29, wherein the proline is L-proline and the concentration of the L-proline in the preparation is from 0.2 to 0.4 M, and wherein the concentration of IgG in the preparation is 6-15% w/v.

36. The preparation of claim 35, wherein the preparation is a liquid preparation that has not been lyophilized and is not lyophilized prior to administration.

37. The preparation of claim 29, wherein the preparation has a pH of about 4.6 to about 5.0, the proline is L-proline and the concentration of the L-proline in the preparation is from 0.2 to 0.3 M, and wherein the concentration of IgG in the preparation is 8-12% w/v.

38. The preparation of claim 37, wherein the preparation is a liquid preparation that has not been lyophilized and is not lyophilized prior to administration.

39. The polyclonal IgG preparation of claim 29, wherein the proline is L-proline and the concentration of the L-proline in the preparation is from 0.2 to 0.4 M, and wherein the concentration of IgG in the preparation is 15-20% w/v.

40. The preparation of claim 39, wherein the preparation is a liquid preparation that has not been lyophilized and is not lyophilized prior to administration.

41. A stable liquid polyclonal IgG preparation, wherein the preparation comprises polyclonal IgG and a stabilizer consisting essentially of proline, wherein the preparation has a pH of about 4.2 to about 5.4, and wherein the preparation is not lyophilized prior to administration.

42. The stable liquid polyclonal IgG preparation of claim 41, wherein the proline is L-proline and the concentration of the L-proline in the preparation is from 0.2 to 0.4 M, and wherein the concentration of IgG in the preparation is 6-15% w/v.

43. The stable liquid polyclonal IgG preparation of claim 41, wherein the proline is L-proline and the concentration of the L-proline in the preparation is from 0.2 to 0.3 M, and wherein the concentration of IgG in the preparation is 8-12% w/v.

44. The stable liquid polyclonal IgG preparation of claim 41, wherein the proline is L-proline and the concentration of the L-proline in the preparation is from 0.2 to 0.4 M, and wherein the concentration of IgG in the preparation is 15-20% w/v.

45. The preparation of claim 39, wherein the concentration of IgG in the preparation is 20% w/v.

Evidence Appendix to Appeal Brief Under Rule 41.37(c)(1)(ix)

Declaration under 37 C.F.R. § 1.132 of Reinhard Bolli, submitted February 9, 2009.

D.L. Tankersley, *Immunological Reviews* 139: 159-172 (1994).

G. Lemm, *Neurology*, 59(Suppl. 6): S28-S32 (2002).

U.S. Food and Drug Administration package insert for Privigen®, Immune Globulin Intravenous (Human), 10% Liquid, issued July, 2007.

U.S. Food and Drug Administration package insert for Hizentra™, Immune Globulin Subcutaneous (Human), 20% Liquid, issued February, 2010.

International Blood/Plasma News: "CSL Behring announced that the U.S. FDA has approved a supplemental Biologics License Application (sBLA) that extends the shelf life of its *Privigen* 10% liquid intravenous immunoglobulin product from 24 months to 36 months," page 12, April, 2010.

M. Cramer et al., *Vox Sang.* 96: 219-225 (2009).

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R.H. Buckley et al., *N. Engl. J. Med.* 325(2): 110-117 (1991).

Related Proceedings Appendix to Appeal Brief Under Rule 41.37(c)(1)(x)

There are no related proceedings identified in this Appeal Brief.

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Dimer Formation in Immunoglobulin Preparations and Speculations on the Mechanism of Action of Intravenous Immune Globulin in Autoimmune Diseases

DONALD L. TANKERSLEY

INTRODUCTION

Since the initial discovery (Imbach et al. 1981) that idiopathic thrombocytopenic purpura (ITP) could be successfully treated with immune globulin intravenous (Igi.v.), over a thousand reports have appeared in the literature suggesting a beneficial effect of Igi.v. in various diseases, many of which are thought to have an autoimmune etiology. Although the persuasiveness of individual reports varies considerably, a substantial body of evidence has been amassed to support the contention that Igi.v. may provide therapeutic benefit, at least in some patients, in certain autoimmune diseases. It should be emphasized, however, that the efficacy of Igi.v. for the treatment of autoimmune diseases has rarely been demonstrated by controlled clinical trials.

In this communication, some of the characteristic features of Igi.v. that may be pertinent to its possible mode of action in the treatment of autoimmune diseases will be described. Although a number of theories have been proposed for the possible efficacy of Igi.v. in such diseases, I will concentrate on the thesis that Igi.v. acts by anti-idiotypic suppression of autoantibodies. This bias is not necessarily because the idea seems particularly likely, but rather because it happens

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Abbreviations used: Ig: immune globulin. Igi.v.: immune globulin intravenous. ITP: idiopathic thrombocytopenic purpura. Id: idiotype.

to be intimately related to investigations from our laboratory dealing with the nature of the IgG dimer.

THERAPEUTIC IMMUNE GLOBULIN PREPARATIONS

Virtually all therapeutic immune globulins (Ig) are prepared from large pools of human plasma by a process of selective precipitation with ethanol at sub-zero temperatures. This process was developed some fifty years ago (Cohn et al. 1946, Oncley et al. 1949) and has survived, with relatively minor modifications, to the present day. One modification, developed by Kistler & Nitschmann (1962), is employed rather widely in several European countries. These fractionation schemes involve the manipulation of five variables (pH, protein concentration, alcohol concentration, ionic strength, and temperature) to selectively precipitate the various proteins of plasma. Fraction II of the Cohn-Oncley process (fraction GG of the Kistler-Nitschmann scheme) consists of essentially pure IgG, with only trace amounts of other plasma proteins such as IgA or IgM. The overall yield of IgG is about 5 grams per liter of plasma, or about 50%. Fraction II (or fraction GG) serves as the starting material for the "standard" intramuscular Ig and for Igi.v.. Ig is prepared by removing residual alcohol from the precipitate by freeze-drying, then dissolving the powder to give a solution of about 16.5% protein. Because this product frequently invokes severe anaphylactic-like reactions when given intravenously, the i.v. route of administration is contraindicated. The precise reasons for these reactions have not been determined, although the presence of IgG aggregates has been implicated (Barandun et al. 1962). IgG aggregates may result in complement activation with release of the anaphylatoxins, C3a and C5a, and a correlation of adverse reactions with complement activation has been observed (Barandun & Isliker 1986). Adverse reactions without significant complement depletion, however, have also been reported (Gerritz et al. 1976). Bleeker et al. (1989) provided evidence that Ig, when administered intravenously to rats, provokes a hypotensive reaction that is mediated by the release of platelet activating factor from macrophages. Contamination of Ig with vasoactive enzymes such as prekallikrein activator or kallikrein has also been suggested as a possible mechanism for adverse reactions (Alving et al. 1980).

A variety of methods have been employed to produce Igi.v. from fraction II or fraction GG. Early approaches involved chemical or enzymic modification of the IgG in an attempt to eliminate the anticomplement activity. Methods included reduction and alkylation of the interchain disulfide bridges of IgG, alkylation of a limited number of lysyl residues with β-propionolactone, or extensive digestion with pepsin or plasmin. Although the resulting preparations were well tolerated, many of the Fc-mediated functions of IgG were substantially diminished.

More recently, preparative methods have attempted to maintain the native structure of the molecule while removing and preventing the formation of aggre-

gates. High molecular weight aggregates can be removed by additional fractional precipitation steps employing polyethylene glycol or ethanol, by ion exchange chromatography, by treatment with small quantities of pepsin at pH 4, or by maintaining the product as a solution at pH 4 throughout its shelf-life. In freeze-dried products, excipients such as human albumin, polyethylene glycol, glycine or sugars (sucrose, maltose, mannitol or glucose) may be added in order to minimize aggregation resulting from lyophilization. As discussed later, these manipulations may affect not only the propensity of the IgG molecules to aggregate, but also the tendency to dimerize. For the most part, presently available preparations are well tolerated, with relatively mild reactions occurring in 1% to 10% of the infusions, depending upon the particular preparation and the underlying disease.

The regulatory agencies of most countries require that each lot of Ig, or Igi.v., shall be prepared from a pool of approximately equal amounts of plasma from not less than 1000 donors. This requirement is easily met, and often substantially exceeded, at the present scale of manufacturing. The rationale for this requirement is an attempt to assure a broad spectrum of antibody specificities to various pathogens. As we shall see, however, this requirement also affects the potential for the Igi.v. to contain anti-idiotypes recognizing other antibodies present either in the Igi.v. itself or in recipients. Under certain conditions, these anti-idiotypes may pair with the complementary idiotype to form a dimeric structure (if both happen to be present in sufficient concentration in the product), or they may interact with complementary idiotypes in a recipient to modify the function of the latter.

IMMUNOGLOBULIN DIMER

Therapeutic Ig preparations contain substantial amounts (up to 40%) of IgG dimer. Early investigations into the nature of this dimeric species suggested that it was composed of two IgG molecules associated end-to-end (Oncley et al. 1947). Later, it was shown that dimerization reflected a reversible temperature-dependent transition, that the rate of dimer formation was exceedingly low (requiring several months to reach equilibrium), and that different Ig preparations differed greatly in their propensity to dimerize (Finlayson et al. 1971). The latter was strikingly demonstrated by the observation that IgG prepared in the laboratory by ammonium sulfate fractionation followed by ion exchange chromatography showed no detectable dimer, even after treatment with ethanol, freeze drying, heating, prolonged storage, or combinations of these procedures. Other investigators (Condie 1980, Suomela 1980) also reported that Ig isolated by ion exchange procedures contained little or no dimer. Because of these observations, it was rather widely accepted that dimerization of IgG molecules in commercial Ig was somehow a result of the ethanol fractionation process itself.

The advent of high-performance exclusion chromatography (HPEC) in the late 1970's greatly facilitated studies of the IgG dimer, which had previously required rather time-consuming analyses by ultracentrifugation or exclusion chromatography on soft gels. With HPEC, the molecular size distribution of Ig preparations could be quantitatively analyzed in minutes, rather than hours or days. In the laboratories at the Center for Biologics Evaluation and Research, it became standard practice to perform HPEC on all Ig lots from various manufacturers. It was soon apparent that these preparations differed substantially in dimer content. Normal Ig lots contained from 25% to 40% dimer, whereas specific Ig preparations contained considerably less (5%–20%). Specific Ig preparations (tetanus Ig, hepatitis B Ig, rabies Ig, etc.) are prepared in the same manner and by the same manufacturers as Ig, differing only in that the plasma donors are stimulated by immunization with the appropriate vaccine and, usually, the plasma pool volumes are smaller (the requirement that each lot of Ig must be prepared from plasma pooled from a minimum of 1000 donors does not apply to specific Ig). To determine whether the low dimer content of specific Ig might be related to the small number of donors, IgG was prepared, by the cold ethanol process as well as by other methods, from plasma obtained from single donors and from pools having known numbers of donors. These experiments led to the surprising discovery that IgG from single-donor plasma contained essentially no dimer ($< 1\%$) and, further, that the dimer content of IgG depended upon the number of donors contributing to the plasma pool (Tankersley et al. 1988).

Additional studies (Tankersley et al. 1988; Roux & Tankersley 1990) to characterize IgG dimer led to the following findings:

(1) IgG dimers are prevalent in Ig prepared from pooled plasma, whereas Ig prepared from single-donor plasma is virtually monomeric. The percentage of dimer increases in proportion to the logarithm of the number of donors.

(2) Dimer content is independent of the method of isolation (alcohol fractionation, ion exchange chromatography, or affinity chromatography). Previous investigators (Finlayson et al. 1971, Condie 1980, Suomela 1980) who reported the absence of dimer in IgG made by ion exchange methods apparently carried out laboratory scale preparations with plasma from one or a few donors, and the absence of dimer was incorrectly attributed to the preparative method. On the other hand, fractionation with ethanol is rarely performed except by commercial fractionators, who normally utilize large volumes of plasma from many donors. Recently a commercial fractionator, Massachusetts Public Health Biologic Laboratories, prepared a lot of botulism Ig by alcohol fractionation of 50 liters of plasma that had been collected, over a period of several months, from only 10 immunized donors. This preparation contained $< 1\%$ dimer.

(3) Dimer content decreases with decreasing pH, increasing temperature, increasing ionic strength, and decreasing IgG concentration. Dimers are almost completely dissociated at pH 4 or below. Thus, little or no dimer is detected in the

Igi.v. manufactured by Miles, Inc. (Gamimune N) which is formulated as a solution at pH 4. However, when 50 different lots of this product were adjusted to pH 7 and stored at 5°C for several weeks before analysis, the dimer content of these lots ranged from 11.4% to 19.7%.

(4) The effect of dilution on dimer content suggests that many distinct equilibria, with differing equilibrium constants, are involved.

(5) $F(ab)'_2$ fragments prepared from multiple-donor Ig dimerize to the same extent as does the IgG in the parent Ig preparation. $F(ab)'_2$ fragments prepared from single-donor IgG do not dimerize.

(6) Neither Fc nor Fab fragments, prepared by plasmin digestion of IgG from large pools, form a dimeric species. Thus, two intact Fabs per IgG molecule are required for dimerization.

(7) Electron microscopy reveals that IgG dimers (as well as $F(ab)'_2$ dimers) consist of pairs in which two arms of each molecule are bound in a reciprocal fashion at or near the distal tips of their respective arms, as previously seen in bona fide idiotype-anti-idiotype complexes.

(8) The propensity of the IgG subclasses to dimerize (IgG3 > IgG1 ≈ IgG4 > IgG2) parallels the reported hinge-region flexibility of the subclasses.

(9) IgG dimerization also occurs in nonhuman species, and is dependent upon the number of donor animals, but the quantitative relationship differs significantly from that of humans. For example, the pooled sera from 49 mice yielded IgG containing only 2% dimer, as compared to 6% in that from 55 humans. IgG isolated from a sample of bovine plasma obtained from a large pool (taken from a railway tank-car) contained 32% dimer. In contrast to single-donor mouse or human IgG, which were devoid of dimer, IgG from a single cow contained 1.3% dimer. These apparent differences between species in the relationship between dimer content and the number of donors may be related to differences in body mass (see below).

Taken together, the foregoing observations strongly suggest that dimerization of IgG is mediated via Id-anti-Id interactions. Although some of these observations might be interpreted as suggesting that the affinity of these interactions are quite low (i.e., the dissociation by heating or lowering the pH, and the requirement for a bivalent interaction), it must be remembered that, because of the large number of different Ids present in solutions of Ig, the concentrations of the individual reactants is very low. Other investigators (Gronski et al. 1988a, 1988b) conducted similar studies on IgG dimer and arrived at the same conclusion, i.e., IgG dimer is an Id-anti-Id complex. By analyzing the kinetics of monomer-dimer equilibria, with the assumption of simultaneous and independent interactions among 10^6 different Ids, these investigators estimated that the association constants were in the range of 10^{10} to 10^{12} M^{-1}. Because the association constant for a monovalent interaction, such as might occur among Fab fragments of these Ids, would be approximately equal to the square root of the bivalent association

constant (i.e., 10^5 to 10^6 M^{-1}), virtually no dimerization of such species would be expected.

A MODEL FOR IMMUNOGLOBULIN DIMERIZATION

What might account for the observation that IgG dimers are prevalent in Ig produced from large plasma pools but are absent in that produced from a single individual? It might be supposed that active mechanisms in an individual serve to control dimer formation. Plausible mechanisms might include the possibility that a particular antibody is capable of suppressing the clonal expansion of B cells bearing surface Ig recognized by that antibody, or alternatively, that dimers are produced by an individual, but are rapidly eliminated from the circulation. Upon reflection, however, it is apparent that such mechanisms cannot account for the absence of dimer in Ig prepared from the plasma of *two* individuals! Also, the fact that dimer is observed in the IgG from a single cow would argue against active mechanisms involving suppression or elimination of dimers.

It appears necessary, therefore, to consider passive mechanisms for an explanation.

An anti-Id may be defined, in the most general terms, as an antibody with a combining site that recognizes, or reacts with, or has affinity for, the combining site of another antibody. Multiple distinct anti-Ids to the same antibody molecule can occur. Alternatively, the same anti-Id may recognize structurally different antibodies. Note the symmetry inherent in this definition. Since the interaction is between the combining sites of two antibodies, there is generally no basis for distinguishing between Id and anti-Id, unless one or the other of the interacting antibodies has a defined immunologic specificity.

In the following discussion, therefore, the term Id-pair will be used to denote two structures which have complementarity, and the term Id-dimer will denote an Id-pair actually engaged in dimerization.

The model which follows is not mathematically precise. Because of the enormous diversity of the immune system and the potential for multiple connectivity within the idiotype network, certain simplifying assumptions are necessary to develop even a crude mathematical model of Id-dimer formation in immunoglobulin preparations. These assumptions, many of which derive from the network theory as proposed by Jerne (1974), and the definitions used in developing a model of Id-dimer formation, will now be described.

The *species diversity* (designated R) of the immune repertoire is defined as the number of different antibodies (hence Ids) that can be produced through the combined mechanisms of V, D, and J segment recombination, H- and L-chain pairing, and somatic mutations arising during B-cell maturation. Since these antibodies have different variable region sequences, they will therefore also differ in idiotype.

It is assumed that the immune repertoire of an individual, at one point in time, is derived through the random selection of m clones from the available species repertoire, where $m << R$. Because the selection of clones is random, some clones may, by chance, be selected more than once, so that the *individual diversity* (designated r) will be less than m. It can be shown, for reasonably large values of m and R (i.e., > 100), that r can be closely approximated by the expression $R[1 - \exp(-m/R)]$. It is further assumed that the immune repertoires of different individuals are independently derived by the process of random selection from the species repertoire as just described. It follows, therefore, that the diversity of Ids in immunoglobulin produced from N donors will be given by $R[1 - \exp(-Nm/R)]$.

It is assumed that all Ids present in an immunoglobulin preparation have equal concentrations. Thus, the concentration of each Id will be given by the total IgG concentration divided by the diversity (i.e., the number of different Ids in the preparation).

It is assumed that the species repertoire includes antibodies recognizing any possible Id. Since antibodies can be produced that recognize virtually any epitope, including idiotopes on other antibody molecules, this assumption does not appear to be unreasonable. It is further assumed that, for each distinct Id in the species repertoire, there exists a small number (x) of distinct anti-Ids, where $x << R/m$ (hence $mx << R$), and that the value of x is the same for each Id. The term x defines the cross-reactivity of the Id. For mathematical simplicity, it will be assumed that $x = 1$; i.e., for each Id in the species repertoire, there is one and only one anti-Id. It should be noted that the model to be described provides essentially the same outcome when values of x greater than one are used, if the value for m is reduced proportionately. In other words, rather than assuming cross-reactivity to a given Id (i.e., $x > 1$), the definition of a "clone" can be broadened to include all antibodies that are idiotypically indistinguishable, i.e., that cross react with a given anti-Id.

The extent to which a given Id-pair will react to form Id-dimer is a function of the concentrations of the interacting Ids and the equilibrium association constant, K_a. It will be assumed that the value for K_a is the same for all of the various Id-pairs. Obviously, if dimer formation is to be significant, the concentrations of the interacting Ids cannot be much less than $1/K_a$. Again, it is recognized that this assumption is a gross oversimplification.

Given the above assumptions, what can be said about the quantity of dimer in IgG preparations as a function of the number of donors? At one extreme, consider a 3% solution (2×10^{-4} M) of IgG from a single donor. Suppose $R = 2 \times 10^7$, and $m = 10^5$. The number of different Ids will be $(2 \times 10^7)[1-\exp(-0.005)] \approx 10^5$ and the concentration of each Id will be $(2 \times 10^{-4}\ M)/10^5 = 2 \times 10^{-9}$ M. Suppose $K_a = 4 \times 10^{10}\ M^{-1}$. Then, if both components of an Id-pair happen to be present, the extent of dimer formation for this pair can be calculated

from the quadratic equation $K_a = [\text{dimer}]/\{[\text{Id}]-[\text{dimer}]\}^2$, giving a value of 89.4%. However, the probability that both components of a particular Id-pair will be present is only ≈ 0.005, so the overall dimer content of the IgG will be $0.005 \times 89.4\% = 0.45\%$. Now, consider a 3% solution of IgG prepared from a pool of 10^4 donors. Again assuming $R = 2 \times 10^7$, $m = 10^5$, the number of different Ids will be $(2 \times 10^7)[1\text{-exp}(-50)] \approx 2 \times 10^7$ and the concentration of each will be $(2 \times 10^{-4}\ M)/(2 \times 10^7) = 10^{-11}$ M. In this case, the extent of dimer formation for a particular Id-pair will be only 23.4% (again, assuming $K_a = 4 \times 10^{10}\ M^{-1}$), but the probability of both components of the Id-pair being included in the IgG preparation is essentially 1. Therefore, the overall dimer content in this case would be 23.4%.

Fig. 1 illustrates this model graphically. In the example shown, $R = 2 \times 10^7$, $m = 1 \times 10^5$, $[\text{IgG}] = 2 \times 10^{-4}$ M, and $K_a = 4 \times 10^{10}\ M^{-1}$. The broken line gives the fraction of Id-pairs present as a function of N, the number of donors. This curve was produced by a computer program that simulated the random selection of $(N \times m)$ clones from a repertoire of size R, then examined the selected clones for complementarity. When plotted in the semilogarithmic form as shown, the shape

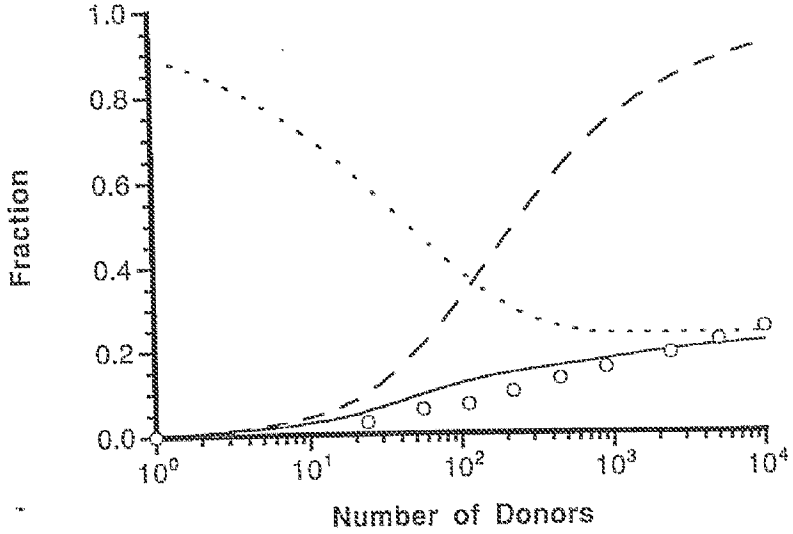

Figure 1. A mathematical model of dimerization in IgG. The dotted line depicts the extent of dimer formation that would occur in an Id-pair interaction having an association constant of $4 \times 10^{10}\ M^{-1}$, when each component of the Id-pair has a total concentration given by $[\text{IgG}]/\{R[1\text{-exp}(-Nm/R)]\}$, where $[\text{IgG}] = 2 \times 10^{-4}$ M (i.e., 30 mg/ml); R (the diversity of the species repertoire) $= 2 \times 10^7$; m (the number of clones produced by an individual) $= 10^5$; and N (the number of donors) is as indicated on the abscissa. The broken line represents the fraction of Id-pairs expected to occur, by chance, in a random selection of $N \times m$ clones from R possible idiotypically distinct clones, where N, m and R are as defined above. The solid line is the product of the broken and dotted lines, and represents the fraction of dimer, as a function of N, that would be predicted by this model. The measured dimer content in IgG samples (at 30 mg/ml) prepared from pools derived from N donors is shown by circles.

of this curve is the same for any values of m or R chosen. Varying these parameters simply alters its position along the x-axis, shifting the curve to the right as the ratio R/m becomes larger. The dotted line is the fraction of each Id-pair that would dimerize, calculated from the concentration of each component of the Id-pair and the value for K_a, again as a function of N. The product of these two curves, shown as the solid line, thus represents the overall Id-dimer content as a function of N. For comparison, the points (circles) give the experimentally determined dimer content in preparations of IgG (at 3% concentration) made from pools with a known number of plasma donors (Tankersley et al. 1988). It is of interest to examine how varying the assumed values for m, R and K_a affects the relationship between the number of donors and dimer content predicted by this model (i.e., the solid line in Fig. 1). First of all, K_a really affects only the "steepness", or slope, of this curve and the plateau value for dimer content at large N. Secondly, as suggested above, altering the ratio R/m produces a shift in the apparent "intercept" of this curve, or its position along the x-axis. The curve shown in Fig. 1, constructed with the ratio R/m = 200, might be considered a reasonable approximation to the experimentally determined relationship between dimer content and number of donors, shown as circles. Since R/m is, essentially, the ratio of the size of the species repertoire as compared to that of an individual, this model suggests that this ratio is about 200 for the human species.

One other aspect of this model deserves comment. Although the x-axis of Fig. 1 is expressed as the number of donors, in terms of the model, each donor is simply providing a "package" of 10^5 IgG clones. As each individual "package" becomes larger (i.e., more clones), fewer donors are needed to achieve the same end. Therefore, if the species repertoire is similar in size for different species, the extent of IgG dimerization for a given number of donors might be greater in large animals (oxen), and less in small animals (mice) than is observed in humans, because the size of the individual repertoire should be a function of the number of mature B cells in an individual, and this number would be related to body mass. With the very limited data available (mice, humans, oxen), this seems to be true. It would be interesting to extend this relationship to include very large mammals such as elephants or whales.The model just presented makes no assumptions regarding either stimulation or suppression of Id by an anti-Id; the simultaneous occurrence of Id-pairs in an individual is considered to be entirely random and the frequency of such occurrences is determined by the relative magnitudes of R and m. The model is also applicable, however, to the supposition that an anti-Id might suppress the expansion and maturation of B cells expressing Id. The only difference, in this case, would be that the frequency of Id-pairs (and hence the amount of dimer) in IgG from a single donor would be zero. Obviously, the production of a particular anti-Id by one individual can have no effect upon the production of Id by another individual, so that the occurrence of Id-pairs in a pool of IgG from multiple donors would again be determined strictly by chance.

There are two important implications that derive from this model. First, Id-pairs (broken curve, Fig. 1) predominate in IgG prepared from large pools with many donors. The observation that dimer content is limited to 25% or so (at a total IgG concentration of 3%) is a result of the very low concentration (i.e., $< 1/K_a$) of individual Id-pairs. Second, the model is compatible with the supposition that the human species immune repertoire is about 200 times larger than the immune repertoire of an individual. Thus, in IgG prepared from thousands of donors, there is a high probability for the occurrence of any given Id (or anti-Id). The ramifications of these implications will be explored in the following section.

MECHANISMS FOR EFFICACY OF Igi.v. IN AUTOIMMUNE DISEASES

Given the present state of knowledge, it is not clear whether a single mechanism might account for the efficacy of Igi.v. in all autoimmune diseases or even in a particular disease. Indeed, in many cases there may be little evidence for efficacy at all! Be that as it may, in general several distinct mechanisms have been proposed. Of these, I will discuss only two; namely, blockade of Fcγ receptors, and anti-idiotypic suppression of autoantibodies.

Fcγ receptor blockade

Perhaps the strongest evidence that Fcγ receptor blockade is responsible for the efficacy of Igi.v. in autoimmune diseases comes from studies on the treatment of patients with acute and chronic ITP (reviewed by Bussel & Pham 1987). In this disorder, platelets become sensitized with autoantibodies and are destroyed by the reticuloendothelial system. Treatment with Igi.v. at dosages of 1 to 2 g/kg body mass often produces a significant increase in platelet count, beginning 2–5 days after Igi.v. infusion and lasting for several weeks, or in some cases even longer. At the dosages given, the IgG concentration in the plasma of patients is substantially increased, often to levels two or three times the pretreatment values. It is thought that this increased level of IgG saturates Fcγ receptors present on cells of the reticuloendothelial system and thereby prevents the destruction of antibody-coated platelets. Two lines of evidence provide support this mechanism. First, low doses of Rh_o(D) immune globulin can produce a therapeutically significant increase in platelets when given to Rh-positive ITP patients, but not to Rh-negative patients (Salama et al. 1986, Bussel et al. 1991). It is assumed that this effect is produced by Fcγ receptor blockade brought about by erythrocytes coated with anti-D. Second, it has been shown recently that infusion of purified Fcγ fragments, at a dosage equivalent (on a molar basis) to those employed with Igi.v., produces an increase in platelets in children with ITP that is comparable to that seen with Igi.v. therapy (Debré et al. 1993). Thus, it seems clear that Fcγ

receptor blockade is a mechanism for the activity of Igi.v. in the treatment of ITP; however, it may not be the only mechanism that is operative. The role of Fcγ blockade in the therapy with Igi.v. of other autoimmune diseases, such as myasthenia gravis (Gajdos et al. 1984), chronic inflammatory polyneuropathy (Vermeulen et al. 1984, Lundkvist et al. 1989) or acquired autoantibodies to antihemophilic factor (Sultan et al. 1984, 1987, Rossi et al. 1989) is much less clear.

Suppression of autoantibodies by antiidiotypes in Igi.v.

The model for IgG dimerization presented earlier suggests that Igi.v. from large plasma pools has a diversity approaching that of the species repertoire, and that many (perhaps most) of these antibodies are one component of an Id-pair. There is, however, one class of antibodies that is absent, or very nearly so. Normally, the immune system does not produce antibodies recognizing endogenous antigens, or self. Exactly how this natural immunological tolerance is brought about during ontogeny is unclear at the present time. Nor is it known why, under certain circumstances, tolerance to a self-antigen can be broken, with the formation of autoantibodies that may result in autoimmune disease. Because Igi.v. is prepared from the plasma of normal, healthy blood donors, the presence of autoantibodies should be rare. However, there is no reason to suppose that anti-Ids recognizing such autoantibodies would be underrepresented. In fact, because one complementary Id (the autoantibody) of a potential Id-pair may be absent, such anti-Ids would be less likely to be encumbered by dimerization. The presence in Igi.v. of anti-Ids to a variety of autoantibodies has been demonstrated (Rossi & Kazatchkine 1989) and, at least in some cases, the anti-Ids were capable of inhibiting the binding of the respective autoantibody to the self-antigen. Although it is conceivable that anti-Ids present in Igi.v. might act by directly suppressing the binding of an autoantibody to the self-antigen (i.e., by dimerization with the autoantibody *in vivo*), the low concentrations of any particular anti-Id, and the limitations dictated by a finite equilibrium affinity constant for dimer formation, would seem to preclude this mechanism. It appears more reasonable that anti-Ids might suppress autoantibody synthesis by binding to surface immunoglobulins on autoreactive B cells and thereby downregulating their maturation, or by a direct or complement-mediated cytopathic effect on B cells and plasma cells secreting autoantibodies recognized by the anti-Id. Such a mechanism might not require a stoichiometric amount of anti-Id, as even a small amount of anti-Id specifically bound to the offending B cells might be sufficient to down-regulate maturation or trigger cytolysis.

SUMMARY

IgG dimers occurring in therapeutic Ig preparations have been characterized as Id-anti-Id, in that the sites of interaction are localized to the distal tips of the

Fab arms. The observation that such dimers are prevalent in Ig or Igi.v. prepared from large plasma pools, but absent in preparations from a single individual, supports the notion that the individual immune repertoire is small with respect to the species repertoire. A crude mathematical model that attempts to relate Id-dimer content to the number of donors is presented. This model suggests that Id-pairs may be much more prevalent in Ig than is reflected by the Id-dimer content, inasmuch as the concentrations of the individual Ids and anti-Ids may limit the equilibrium level of dimer. The model further suggests that the antibody diversity in Ig derived from thousands of donors may be representative of the species repertoire; hence, virtually all specificities, including anti-Id specificities, will be included. Because Ig is derived from normal healthy donors, it should be relatively free of pathogenic autoantibodies. However, there is no reason to suspect that anti-Ids to such autoantibodies would not occur, and indeed the presence of such anti-Ids has been demonstrated. Several mechanisms have been proposed by which such anti-Ids might ameliorate autoimmune disease. They may directly inhibit the binding of autoantibody to its target antigen, or they may target for destruction those cells expressing or secreting autoantibody.

It may well be that anti-Ids play no role in the mechanism of action of Igi.v. in autoimmune disease. Such appears to have been demonstrated, for example, in the treatment of ITP. There is an obvious need for additional studies in order to elucidate the mechanism of action of Igi.v. in various autoimmune diseases. Experimental animal models of autoimmune disease, such as the mouse model for systemic lupus erythematosus (Mozes et al. 1993), might be very useful in this regard.

Finally, it needs to be emphasized that the usefulness of high doses of Igi.v. in many autoimmune diseases remains to be established by controlled clinical trials. Because Igi.v. is a limited resource, and one which cannot be produced through biotechnological advances (at least in the foreseeable future), its widespread use should be restricted to the treatment of diseases for which efficacy has been demonstrated. To do otherwise might deprive appropriate patients of a valuable therapy.

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Composition and properties of IVIg preparations that affect tolerability and therapeutic efficacy

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Article abstract—The use of intravenous immune globulin (IVIg) has increased significantly in the past decade, benefiting a wide variety of immune diseases. Seven different formulations of IVIg are now licensed in the United States. Although all contain pooled IgG, there are differences in their production and composition that affect their efficacy, tolerability, and side-effect profile. Important variables include concentration, volume, osmolality, sodium, and sugar content. This article reviews what is known about the composition and properties of the various IVIg formulations that might affect the therapeutic outcome.

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Intravenous (human) immunoglobulin (IVIg), which was introduced in the 1950s as replacement for patients with congenital antibody deficiencies, has since established its place in the treatment of a wide variety of immune diseases.[1] The Igs are precipitated from human plasma using fractionation methods developed more than five decades ago by Cohn-Oncley or Kistler-Nitschmann. Because they were administered intramuscularly, usually at doses ranging from 25 to 100 mg/kg of body weight, the injections were not only painful but also were limited by the amount of Ig that could be given comfortably and safely. Nevertheless, even at these doses, replacement therapy was considered lifesaving, extending the lives of patients with primary antibody deficiency.[2] Of particular relevance to the evolution of our current thinking, these doses both reduced the number of infections that patients developed and appeared to modify the course of associated conditions such as immune cytopenias, skin rashes, and alopecia totalis.[3] Perhaps these were the first indications that Ig replacement also had immunomodulatory properties.

The early preparations could not be given intravenously because they contained aggregates and other impurities capable of activating complement and causing severe reactions, including anaphylaxis.[4] To reduce these reactions, a series of additional processing modifications were introduced, with the goal of stabilizing and purifying IgG and preventing aggregate formation.[5] The initial attempts at processing primarily used chemical modifications or proteolytic digestion, which reduced the incidence of untoward reactions but compromised the biological activity and serum half-lives.[5]

Later improvements in processing resulted in preparations with high levels of intact IgG, normal distribution of IgG subclasses, and small concentrations of dimers. In the early 1980s, these new products, suitable for intravenous administration, became commercially available. Intravenous administration of Igs allowed the use of larger doses without the pain associated with intramuscular injections. Administration of larger doses proved even more effective in preventing infection and maintaining lung function in patients with primary antibody deficiency diseases.[6] Over the past decade, a number of intravenous preparations have been further modified by specific virus inactivation or removal steps that made them safer. Seven polyclonal IVIg are now approved for use and marketed in the United States, along with four additional hyperimmune products suitable for intravenous use for specific indications such as infection by cytomegalovirus, hepatitis B, respiratory syncytial virus, and Rh prophylaxis.

The differences in basic fractionation methods and the addition of various modifications for purification, stabilization, and virus inactivation and removal resulted in products that were significantly different from each other with respect to chemical structure, antibody content, subclass distribution, and electrophoretic profile (table). Some attempts at standardization, e.g., by a WHO Expert Committee on Biologic Standardization,[2] have mandated that Ig must be as unmodified as possible, must maintain its biological function (opsonic activity, complement fixation, Fc receptor binding), must contain certain levels of specific antibody, and must meet accepted safety standards. However, IVIg preparations are not routinely tested for antibody titer, affinity, or function, such as opsonization,[7,8] which might be affected by the method of purification and might vary among preparations. For example, when commercial

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Table *Comparison of different IVIg*

Category	Veno-S (5%)	Veno-S (10%)	Gammagard S/D	Iveegam EN	Polygam S/D	Gamimmune N S/D 10%	Gammar P.I.V.	Immune globulin intravenous (human)
Manufacturer	Alpha Therapeutic Corp.		Baxter Corp./ Hyland Immune Division	Immuno-US	Baxter Corp./ Hyland Immune Div.; Distrib. by American Red Cross (ARC)	Bayer	Aventis Behring	ZLB Bioplasma
Method of preparation (including viral inactivation)	Cold alcohol/fractionation, PEG/bentonite fractionation, ion-exchange, chromatography, solvent detergent treatment		Cohn-Oncley, ultrafiltration, ion-exchange, chromatography, solvent detergent treatment	Cold ethanol, PEG, trypsin	Cohn-Oncley, ultrafiltration, ion-exchange, chromatography, solvent detergent treatment	Cohn-Oncley, pH 4.25, solvent detergent treatment	Cohn-Oncley pasteurization, ultrafiltration	Kistler-Nitschmann; pH 4.0 + trace pepsin
Form	Liquid		Lyophilized	Lyophilized	Lyophilized	Liquid	Lyophilized	Lyophilized
Shelf-life	24 months		27 months	24 months	27 months	36 months	24 months	24 months
Reconstitution Time	Liquid solution		<5 minutes at room temp.; >20 minutes if cold	≤10 minutes at room temp.	<5 minutes at room temp.; >20 minutes if cold	Liquid solution	<20 minutes	Several minutes
Recommended concentration	5%	10%	5%	5%	5%	10%	5%	3%
Recommended infusion rate	3 mL/kg/hr	3 mL/kg/hr	4 mL/kg/hr	2 mL/min	4 mL/kg/hr	4.8 mL/kg/hr	3.6 mL/kg/hr	2.5 mL/min
Time to infuse 70 g (1 g/kg)	7 hr	3.5 hr	5.3 hr	12 hr	5.3 hr	2.3 hr	5.8 hr	16 hr
Composition								
Sugar content	5% D-Sorbitol		2% glucose	5% glucose	2% glucose	Sugar-free	5% sucrose	5% sucrose
Sodium content	1.3 mEq/L	<1 mEq/L	0.85% at 5% concentration	0.3%	0.85% at 5% concentration	Trace	0.5%	Up to 0.9%, depending on diluent
Osmolality	300 mOsm/L		5% 636 mOsm/ L, 10% 1,250 mOsm/L	≥240	5% 636 mOsm/L, 10% 1,250 mOsm/L	274 mOsm/L	5% 309 mOsm/ L, 10% 600 mOsm/L	In sterile water (mOsm/L): *3%* 192 *6%* 384 *12%* 768 In normal saline (mOsm/L): *3%* 498 *6%* 690 *12%* 1074
pH	5.2–5.8		6.8	6.4–7.2	6.8	4.25	6.8	6.6
IgA content	15.1 μg/mL	20–50 μg/mL	<3.7 μg/mL	<10 μg/mL	<3.7 μg/mL	270 μg/mL	<25 μg/mL	720 μg/mL

Ig products were compared for antibody activity to *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and group B *Streptococcus*, they were found to have differences and inconsistencies among batches and formulations, with some of the products showing reduced opsonization activity.[9-11] Different procedures have also been shown to affect Fc receptor activity[12] or complement fixation,[13] which can influence the protective effects of IVIg in patients with antibody deficiency.[14] These factors may significantly affect clinical outcome in treated patients.

Production of IVIg. IgG was first isolated from human plasma by the Cohn-Oncley process (cold ethanol fractionation) in the early 1940s.[15] IVIg are prepared from plasma pooled from hundreds to thousands of donors. Most production processes begin with sequential precipitation and fractionation to isolate IgG from other plasma proteins. The material is subjected to freeze-drying to remove the ethanol and produce stable intermediates. Freeze-drying in the presence of ethanol promotes formation of insoluble IgG aggregates. The IgG concentrates from initial fractionation are then subjected to additional

processing to produce material suitable for intravenous administration. Earlier methods that involved treatment with proteolytic enzymes gave way to chemical modification in an attempt to preserve the integrity of the IgG molecule, reduce aggregate formation, and eliminate anticomplement activity. Further modifications led to improved products, higher purity, improved stability, and a normal distribution of IgG subclasses.

The different processing methods yield products that differ in their content of IgG monomers, dimers, and polymers, and that include different amounts of contaminants such as albumin. The various processing steps and harshness of treatment also contribute to reduced integrity of the IgG molecule and loss of biological function. These differences can affect a product's efficacy as well as the incidence of adverse events.

Properties of IVIg preparations. *Liquid vs. lyophilized IVIg preparations.* The manufacturing process determines whether the final product is in liquid or lyophilized form. Liquid preparations are more convenient, easier to use, and associated with fewer adverse events. In ready-to-use form, liquid preparations avoid preparation time and delays for patients. They also avoid wastage if a patient does not show up for treatment, because lyophilized preparations usually need to be prepared before the patient arrives.

IVIg concentration. Products that are given at higher concentrations reduce the volume load of treatment, an important consideration for certain patient populations. For example, a 70-kg adult receiving 2 g/kg body weight would receive 1,400 mL of a 10% solution compared with 2,800 mL of a 5% solution. Simply concentrating certain products by reconstituting them in a smaller volume of fluid can be risky because the osmolality of the solution increases and may trigger significant adverse events, such as renal complications or thromboembolic episodes.

Viral inactivation and pathogen safety. The manufacturing process for IVIg minimizes the risk that the product can transmit an infectious disease. Manufacturers have imposed safety standards for viral pathogens that document the capacity of the manufacturing process to remove or inactivate viruses. Plasma testing, of both individual donations and manufacturing pools, is the initial step in ensuring that source material is free of high levels of clinically significant viruses. Accepted viral inactivation steps include treatment with a solvent detergent, polyethylene glycol, trypsin, pasteurization, nano-filtration, low pH, and depth filtration. The traditional calculation of overall reduction in clinically significant viruses is based on the sum of reductions achieved by each of the production steps.[16] Steps that act through independent mechanisms to inactivate or remove viruses are considered complementary and increase overall safety by providing the widest possible safety margin against known and unknown viruses. Treatment with Caprylate (caprylic acid) in particular, a naturally occurring octanoic fatty acid, is effective at inactivating viruses under varied conditions of pH, temperature, and protein levels.[17,18] It is less harsh and more rapidly acting than solvent detergent, resulting in improved viability.

Storage and stability. IVIg preparations also vary in their stability and their ability to be stored at room temperature. The shelf-life for currently licensed IVIg formulations ranges from 24 to 36 months. Expired preparations should not be used to treat patients.

Composition of IVIg preparations. IVIg preparations vary in their composition, depending on the starting materials and manufacturing process, modifications, and stabilizers.[19] The following sections discuss the major and minor components or determinants of the various IVIg preparations that might affect their properties.

Major components or determinants. *Sugar content.* Various sugars such as sorbitol, glucose, and sucrose are added to some preparations as a stabilizer, preventing aggregate formation. The major problem associated with sugar content is the incidence of significant adverse events, particularly acute renal failure or insufficiency. Although these complications are rare, the CDC reported that 90% of the IVIg-associated adverse renal events in the United States occurred with sucrose-containing IVIg preparations.[20]

Sodium content. Sodium content varies widely in the different IVIg preparations, ranging from trace amounts to 0.9% concentrations. The sodium content determines the osmolality of the infused solution, which can affect tolerability and occurrence of adverse events. A high sodium content is particularly problematic in patients with hypertension, renal impairment, or those on a low-salt diet. Caution should be exercised when lyophilized preparations are reconstituted to higher concentrations in an attempt to reduce volume load, because that may increase the salt concentration and osmolality to unacceptable levels.

Osmolality. Osmolality is the count of the total number of osmotically active particles in a solution and is equal to the sum of the molalities of all the solutes present in a solution. In IVIg solutions, the major contributors to osmolality include sodium, sugars, and other solutes or amino acids that may be present. Physiologic osmolality is 280–296 mOsm/kg of water, but IVIg solutions may range in osmolality from physiologic values to greater than 1,000 mOsm. Sugar-stabilized products tend to have a higher osmolality than sugar-free (glycine) preparations. Hyperosmolar solutions may cause fluid shifts when infused intravenously, thus contributing to hemodynamic changes and the occurrence of infusion-related adverse events. In reconstituting lyophilized preparations, attention should be paid to the final osmola-

lity of the reconstituted product so as to minimize such side effects.

Minor components or determinants. *pH.* Many IVIg products have a final pH that is close to neutral (a pH of 7.0–7.5) or in the range of 6 to 7. However, the optimal pH to prevent aggregation is 4.0 to 4.5. As a consequence, when an IVIg preparation has a pH greater than 4.5, agents must be added to maintain stability and prevent aggregation. Low-pH preparations are instantaneously neutralized by the buffering capacity of blood on infusion.

IgA content. Patients with selective IgA deficiency and IgG or IgE anti-IgA antibodies are at risk for developing a reaction, rarely anaphylactic, after receiving IVIg. The NIH consensus conference on IVIg did not recommend screening patients who receive IVIg for anti-IgA antibodies,[19] but screening is often done nevertheless. Different IVIg preparations vary in their content of IgA, although using a preparation that is low in IgA may not prevent a reaction.

Isohemagglutinin antibodies. Preparations of IVIg contain low titers of anti-A, anti-B, anti-C, and anti-E blood group antibodies. Passively acquired, these antibodies may be detectable, transiently, in post-treatment direct and indirect antiglobulin tests.[21] To date, there are no known reports that these antibodies in (nonhyperimmune) IVIg preparations were associated with hemolysis. The European Union Pharmacopeia mandates that anti-A and anti-B titers should be less than 1:64 in IVIg preparations.

Other antibodies. IVIg preparations may differ in antibody titers to organisms, such as echovirus[22] or *Staphylococcus epidermidis*,[23] that may affect the outcome in immune-deficient patients. Specific antibody levels may also affect the efficacy of IVIg preparations in autoimmune or allergic diseases, through anti-idiotypic interactions, or through their binding to endotoxin, superantigen, or cytokines in the affected patients.

Tolerability of IVIg preparations. Tolerability is a measure of the ability of a patient to receive a formulation of IVIg without infusion-related adverse events. Tolerability varies markedly among IVIg preparations and patient populations. Older patients, for example, or those with cardiovascular disease, may be less tolerant of preparations that have high osmolality or require larger volumes.

The rate of infusion is an important variable that could influence the occurrence of adverse effects, including thromboembolism.[24,25] Current recommended infusion rates fall within the range of 0.03 to 0.13 mL/kg/min, depending on the preparation. As a rule, preparations that are high in osmolality or sugar content have slower recommended infusion rates. The more rapid the infusion rate and the higher the concentration, the shorter the length of time it would take to infuse a particular dose of IVIg.

Summary. IVIg differ in their content, production, composition, and properties. These differences can influence the tolerability, rate of infusion, stability, associated adverse effects, and efficacy of the various IVIg preparations. These factors must be considered when treatment is ordered for patients who require IVIg therapy.

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ORIGINAL PAPER

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Stability over 36 months of a new liquid 10% polyclonal immunoglobulin product (IgPro10, Privigen©) stabilized with L-proline

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Background and Objectives IgPro10 (Privigen©) is a new liquid intravenous immunoglobulin (IVIG) product that is formulated with 250 mM L-proline at pH 4·8. A 3-year study was performed to assess its stability.

Materials and Methods Physicochemical, biological and immunological parameters of Privigen were assessed during storage under controlled conditions over up to 36 months at 25°C.

Results Privigen was shown to be stable after storage for 3 years at room temperature (25°C). Of all parameters tested, only a few showed changes during storage. The appearance of the solution complied with the specifications given by the European Pharmacopeia over the full study period, with a single exception. The IgG fraction in Privigen displayed high purity (≥ 98%), which did not change during storage over 36 months. No relevant amounts of aggregated IgG molecules were formed in Privigen samples and the ratio between monomers and dimers shifted slightly towards the equilibrium over time. Testing of reference antibody contents and the Fc function demonstrated that the biological activity and effector functions of Privigen were preserved over the full study period of 36 months.

Conclusion Thirty-six months room temperature stability of Privigen was achieved at pH 4·8 with an innovative formulation containing the physiological stabilizer L-proline.

Key words: dimer, intravenous immunoglobulin, IgPro10 (Privigen), L-proline, stability.

Introduction

Immunoglobulins for intravenous administration (IVIG) are used for the treatment of primary and secondary immunodeficiencies and autoimmune diseases [1–4]. IVIGs are available in two formulations, liquid and lyophilized. Liquid IVIG formulations have several advantages over lyophilized ones. They are easier to use and avoid preparation time delays and wastage of material. However, liquid IVIG formulations have limited shelf-lives [5,6]. Long-term storage of liquid IVIG formulations has therefore required the use of refrigerated conditions until now. Storage of liquid IVIGs at room temperature is beneficial in terms of ease and cost of storage and because the IVIGs would be ready to use without the need for temperature adjustment before infusion. Here, we describe the results of a 3-year study showing the stability and activity of a new liquid IVIG IgPro10 (Privigen©) upon extended storage at 25°C.

One of the most challenging tasks when developing a new formulation of liquid IVIG is addressing the physical and chemical instabilities of IgG, such as aggregation, fragmentation and oxidation [7–9]. Aggregation occurs when proteins bind to each other in order to minimize thermodynamically unfavorable interactions between solvent and exposed hydrophobic residues of proteins [7]. Various factors, such as IgG concentration, pH of the solution, temperature and agitation, might induce aggregation [9]. Fragmentation often

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occurs as a result of deamidation, oxidation or hydrolysis. These processes are affected by the pH of the solution, temperature and exposure to light. Oxidation may also be responsible for the yellow discoloration of IVIG solutions and is therefore important in IVIG formulation development. Even minor alterations in the structure of IgG may result in changes in antigen-binding specificity or in the functional aspects of an antibody, resulting in reduced activity, reduced solubility and altered immunogenicity [7,10]. Therefore, maintaining stability of IgG is crucial for preservation of functionality, efficacy and tolerability of IVIGs.

Another consideration in IVIG formulation development is idiotype-anti-idiotype dimers, which form when IgG molecules have variable sites that recognize each other. The binding occurs through electrostatic and/or hydrophobic interactions and is reversible. Thus, an equilibrium between dimers and monomers exists. Formation of idiotype-anti-idiotype dimers is mainly dependent on the IgG concentration, the pH of the solution, the donor pool size, interaction time and the temperature [11]. Presence of high levels of IgG dimers has been associated with mild and transient adverse reactions including headache, fever and flushing [8]. Dimers have also been implicated in development of hypotension in a rat model [12,13].

Privigen is a new ready-to-use formulation of polyvalent human IgG for intravenous administration. The sterile 10% IgG solution is stabilized with 250 mM L-proline at pH 4·8, has a low sodium content and does not contain any preservatives. As an amphiphilic compound, L-proline was shown to provide idiotype-anti-idiotype dimer control [14], while maintaining superior stability of IgG at room temperature, allowing long-term storage. Furthermore, L-proline was shown to have no neurological effects in rats, even at doses much higher than those administered with IVIGs [14]. Here we show that after 3 years of storage at room temperature, aggregation, dimerization and degradation of IgG in Privigen are minimal and the activity of specific antibodies is preserved.

Methods

Manufacturing process

Privigen is prepared from large donor pools and represents the antibody spectrum present in the donor population. The IgG fraction from plasma is purified by a combination of cold ethanol fractionation, octanoic acid precipitation and anion exchange chromatography (see Fig. 1). Fractionation and precipitation for the Privigen lots of the present studies were performed by the method according to Kistler and Nitschmann [15]. Alternatively, the plasma for Privigen may also be fractionated by the method according to Cohn [16].

The manufacturing process employs methods with three different mechanisms of viral clearance: virus inactivation by incubation at pH 4, virus removal via size exclusion filtration and partitioning.

Packaging and storage

Privigen was filled into 50 ml and 100 ml infusion bottles of glass Type I and sealed with chlorobutyl rubber stoppers

Process Flow Chart	Purpose	IgG purity	Proteolytic activity[1]
Plasma pool	• Represents antibody spectrum representative of the donor population	< 15%	–
Cold Ethanol Fractionation	• Isolation of crude immunoglobulin fraction	> 50%	741
Octanoic Acid Precipitation + Clarification	• Purification of immunoglobulins • Clarification of the protein solution	> 90%	1·9
Anion Exchange-Chromatography	• Polishing of the IgG fraction; removal of non-IgG immunoglobulins	~ 99%	< 0·15

[1]Proteolytic activity in ncat/g IgG

Fig. 1 Manufacturing process flow chart. Purpose of process step and its contribution to immunoglobulin G (IgG) purification and removal of proteolytic activity. The IgG fraction from plasma is purified by a combination of cold ethanol fractionation, octanoic acid precipitation and anion exchange chromatography.

Table 1 Testing schedules for stability studies with Privigen

Storage condition	Schedule	Test time-point (months)								
		0	3	6	9	12	18	24	30	36
25°C	A	X							X	X
	B	X	X	X	X	X	X	X	X	X

coated with Teflon® (FluroTec®, West Pharmaceutical Services, Eschweiler, Germany). The Privigen bottles used in the stability studies were stored in the dark to simulate secondary packaging.

Stability studies with five process validation lots and two scale-up lots of Privigen were performed under long-term storage conditions. Four lots in the 50 ml fill size and three lots in the 100 ml fill size were stored at 25 ± 2°C.

Bottles in all studies were stored in the horizontal position in order to maintain product contact with the rubber stopper. The stoppers complied with the European Pharmacopoeia requirements for Type I rubber closures for containers for aqueous preparations for parenteral use.

Stability assessment

Stability studies were performed in accordance with current guidelines of the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) [17,18]. The requirements for a polyclonal immunoglobulin (IVIG) product are defined in monographs such as European Pharmacopoeia (01/2008:0918) and in US 21 Code of Federal Regulations 640·104. According to these monographs, immunoglobulin products need to fulfil compendial or Code of Federal Regulations requirements at the end of their shelf-life at a defined storage temperature. In the current publication, 'stable' indicates a product in compliance with these rules.

Testing schedules for stability studies are shown in Table 1. Purity at 25°C was measured according to schedule A. All other parameters tested were measured according to schedule B. Test time-points were selected based on requirements in the ICH guideline Q1A(R2) [18].

Acetate electrophoresis

The purity of the Ig fraction was measured by acetate electrophoresis. Proteins were separated according to their electrophoretic mobility on a cellulose acetate membrane. After staining with Ponceau Red S, the relative amount of each fraction was calculated by densitometric evaluation of the area under the curve. The method allows the determination of the five major classes of proteins in human plasma or serum (albumin, α1-, α2-, β- and γ-globulins).

Functionality (Fc function)

Human red blood cells of the blood group O were coated with rubella antigen. The immunoglobulin sample (antibodies) to be tested was added and the mixture was incubated. After addition of guinea pig complement, the lysis of the red blood cells was induced through complement activation by antigen–antibody complexes on the cell surface. The kinetic of haemolysis was measured photometrically at 541 nm and compared to a reference preparation.

High-performance liquid chromatography

Levels of IgG monomers and dimers, aggregates and fragments were determined by size-exclusion high-performance liquid chromatography (SE-HPLC) on a size-exclusion chromatography column. Aggregates, dimers, monomers and fragments were separated on a hydrophilic silica size-exclusion resin using a phosphate buffer as mobile phase. Ultraviolet detection was done at 280 nm. The relative amount of each fraction was calculated from the peak areas under the curve.

Enzyme immunoassay

The titers of specific antibodies against hepatitis B surface antigen (anti-HBs) were determined by enzyme immunoassay. DADE Behring Enzygnost test, a one-step, solid-phase enzyme immunoassay for the quantitative verification of anti-HBs based on the sandwich principle was used. Microwell plates were coated with hepatitis B and incubated with sera. Peroxidase-marked hepatitis B was added. Following removal of the unbound reactant, the bound enzyme activity of the conjugate was determined by the color reaction caused by the substrate (tetramethyl benzidine). Anti-HBs concentrations were determined by comparing the absorption values with controls calibrated against a World Health Organization standard.

Nephelometric assay

Polystyrene particles coated with streptolysin O were mixed with samples containing streptolysin O antibodies. Agglutination was measured by a photo detector. The assessment was based on a standard curve prepared with a commercial standard.

Visual aspect

The appearance of the solution (clarity, discoloration, opalescence, particulate matter and flake formation) was assessed qualitatively by comparison of the product using reference backgrounds.

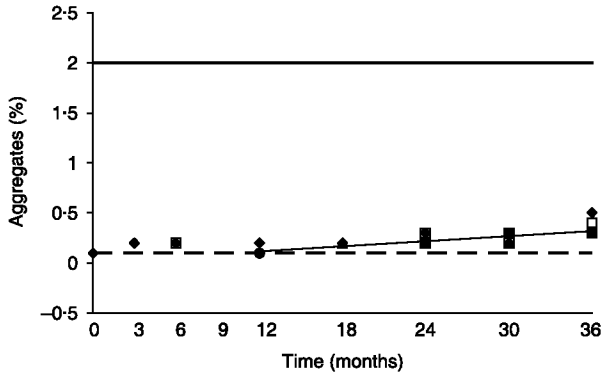

Fig. 2 Change in aggregate levels over time upon storage for 36 months at 25°C. Samples were taken at indicated times for analysis of aggregate levels by high-performance liquid chromatography (HPLC). Results for individual lots are shown. Black symbols represent 50 ml lots and white symbols represent 100 ml lots. Statistical trend analysis was performed as described in Methods, and a significant linear trend ($P < 0{\cdot}05$) was found for 1 of 7 lots. The thick solid line represents the specification limit of aggregate levels and the dashed line represents the detection limit of the assay.

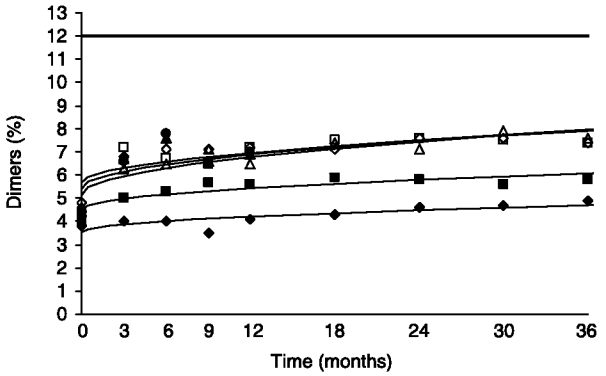

Fig. 4 Change in dimer levels over time upon storage for 36 months at 25°C. Samples were taken at indicated times for analysis of dimer levels by high-performance liquid chromatography (HPLC). Results for individual lots are shown. Black symbols represent 50 ml lots and white symbols represent 100 ml lots. Statistical trend analysis was performed as described in Methods, and significant linear trends ($P < 0{\cdot}05$) were found for 5 of 7 lots. The thick solid line represents the specification limit of dimer content.

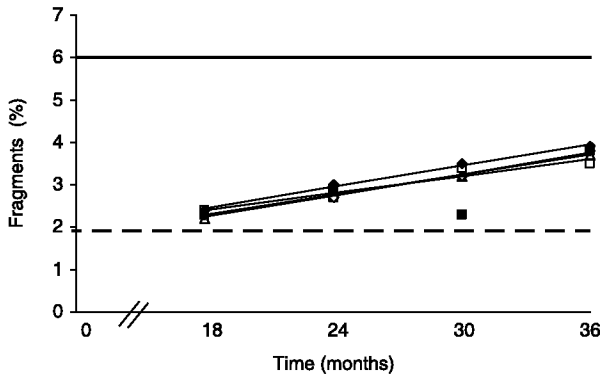

Fig. 3 Change in fragment levels over time upon storage for 36 months at 25°C. Samples were taken at indicated times for analysis of fragment levels by high-performance liquid chromatography (HPLC). Fragment levels in samples taken at 0, 3, 6, 9 and 12 months were below the detection limit of 1·9% (dashed line). Results for individual lots are shown. Black symbols represent 50 ml lots and white symbols represent 100 ml lots. Statistical trend analysis was performed as described in Methods, and significant linear trends ($P < 0{\cdot}05$) were found for 4 of 7 lots. The thick solid line represents the specification limit of fragment levels and the dashed line represents the detection limit of the assay.

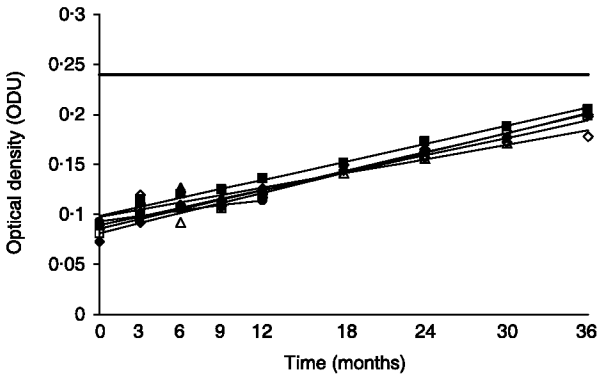

Fig. 5 Change in optical density over time upon storage for 36 months at 25°C. Samples were taken at indicated times for analysis of optical density by spectrophotometry. Results for individual lots are shown. Black symbols represent 50 ml lots and white symbols represent 100 ml lots. Statistical trend analysis was performed as described in Methods, and significant linear trends ($P < 0{\cdot}05$) were found for 6 of 7 lots. The thick solid line represents the specification limit of optical density. ODU: optical density units.

Spectrophotometry

In addition to the above named qualitative method, the discoloration of the sample was specifically determined by measuring its optical density at 350 nm. The optical density at a second wavelength (500 nm) was subtracted in order to correct for turbidity [19]. The resulting number is a measure for the intensity of the yellow-brownish coloration of the sample. Holmium oxide filters or holmium perchlorate solutions were used as reference materials.

Depiction and analysis of results (see Figs 2–7)

Data were statistically evaluated according to ICH Q1E [20]. Each single value was checked for compliance with the specified limit or range given as acceptance criteria. For the results of each quantitative parameter and lots with at least

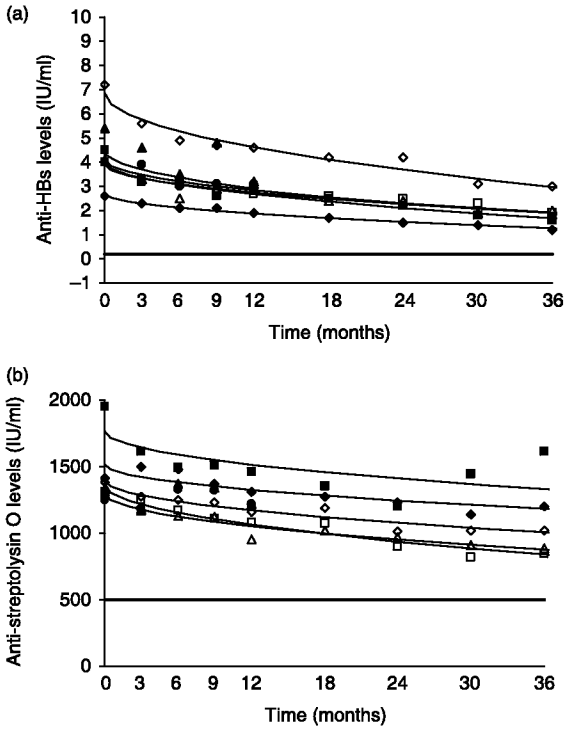

Fig. 6 Change in anti-hepatitis B surface antigen (anti-HBs) (a) and anti-streptolysin O (b) levels over time upon storage for 36 months at 25°C. Samples were taken at indicated times for analysis. Results for individual lots are shown. Black symbols represent 50 ml lots and white symbols represent 100 ml lots. Statistical trend analysis was performed as described in Methods, and significant linear trends ($P < 0{\cdot}05$) were found for 6 of 7 lots for anti-HBs antibodies and 5 of 7 lots for streptolysin O antibodies. The thick solid lines represent the specification limits for the streptolysin O (500 IU/ml) and anti-HBs (0·2 IU/ml) antibody levels, respectively. Similar results were seen when other specific antibodies were assessed (data not shown).

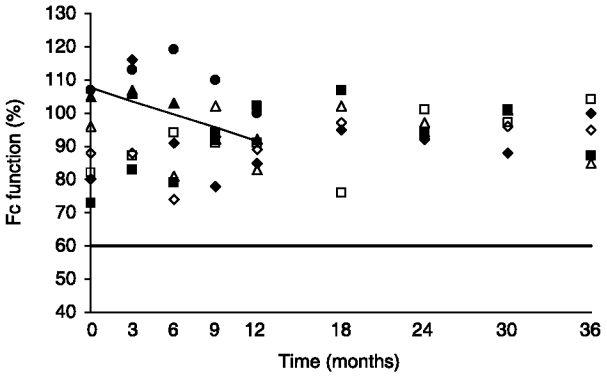

Fig. 7 Change in Fc function over time upon storage for 36 months at 25°C. Samples were taken at indicated times for analysis. Results for individual lots are shown. Black symbols represent 50 ml lots and white symbols represent 100 ml lots. Statistical trend analysis was performed as described in Methods, and a significant linear trend ($P < 0{\cdot}05$) was found for 1 of 7 lots. The thick solid line represents the specification limit of Fc function.

four consecutive values, a statistical trend analysis was performed by determining the *P*-value of the slope (*x*-variable: 'time') based on the 95% confidence interval. A *P*-value lower than 0·05 (probability of error) indicated a statistically significant trend. Non-linear datasets were mathematically transformed to be able to calculate the *P*-value. In Figs 2–7, data of individual lots and filling sizes are depicted together with their respective regression line. Data below detection limits of the respective method were excluded from any calculation. Specifications and detection limits are given for each parameter.

Results

Privigen exhibited good stability during storage at 25°C. The aggregate content was below the detection limit of 0·1% up to 9 months in five of seven lots. By 36 months, the levels increased to between 0·3% and 0·5% (Fig. 2), but remained well below the specified limit of 2·0%. Levels of fragments were below the detection limit (1·9%) up to 12 months in all seven lots and slightly increased at later time-points, up to 3·9% after 36 months of storage, remaining significantly below the specification limit of 6·0% (Fig. 3). The purity (IgG) and the monomer/dimer levels of Privigen stored up to 36 months at 25°C remained high with 98% and 96%, respectively (data not shown). The equilibrium between monomers and dimers shifted slightly over the course of the study, most notably in the first 6 months, after which the levels remained relatively stable (Fig. 4). Initial dimer levels ranged between 3·8% and 4·8%. After 36 months of storage, dimer levels ranged from 4·9% to 7·6%, which is below the specification threshold of 12%.

The appearance of Privigen was a clear or slightly opalescent and colorless or pale yellow solution with an exception of a single 'fail' result in one lot after 36 months of storage. The 'fail' was attributed to a yellow discoloration of the product, while clarity, opalescence, particulate matter and flake formation passed the test. The quantitative measurement of the grade of discoloration by optical density resulted in values from 0·073 to 0·092 optical density units (ODU) at day 0 to 0·178–0·205 ODU after 36 months of storage (Fig. 5), never exceeding the specification limit of 0·240 ODU.

The levels of specific antibodies were measured in order to assess the effect of storage conditions on antibody titers. Antibodies against hepatitis B surface antigen and those against streptolysin O decreased over 36 months compared to day 0, remaining above the specified minimum limit of 0·2 IU/ml and 500 IU/ml, respectively (Fig. 6a,b). Similar results were obtained for other specific antibodies (data not shown).

Function of the Fc antibody region was measured by the method specified in the European Pharmacopeia. All results

were above the limit of 60% during the course of the studies with a range of 73–107% at study start and 85–104% after 36 months (Fig. 7).

Discussion

The stability of IVIG preparations depends on the purification process used, their formulation (liquid or lyophilized), storage temperature and conditions, and, for liquid formulations, the IgG concentration, pH and the excipients included in the solution [7]. Liquid preparations are more convenient to use than lyophilized ones as there is no need for reconstitution [5]. However, currently available commercial liquid IVIG preparations have limited shelf-lives at room temperature [5,21]. Here we show that Privigen, a new ready-to-use 10% liquid IVIG preparation formulated with 250 mM L-proline at pH 4·8, is stable for up to 36 months at room temperature.

Deterioration in stability of IgG and/or a significant increase in IgG dimer content may result in decreased efficacy and tolerability of IVIG [8]. To improve the stability and to control the dimer content of IVIG solutions, an optimal combination of pH adjustment and the addition of L-proline as a stabilizer were found.

The purity of the protein is also a major determinant of stability of liquid protein pharmaceuticals, because the presence of even trace amounts of proteases or other contaminants can potentially affect protein stability [9]. The fractionation procedure for Privigen results in highly purified and intact IgG with a broad range of specific antibodies.

Temperature is one of the most important factors affecting protein stability by causing heat denaturation as well as enhancing chemically induced denaturation, which may then result in degradation and aggregation of the protein [9]. Storage conditions of different products depend on the stability of the product, which must be in compliance with international guidelines [17,18]. Therefore, the stability of a product is indicated by its stated shelf-life. Highly concentrated liquid IVIG formulations are more prone to aggregation and are therefore less stable at room temperature than those with lower IgG concentrations [11]. IVIG formulations containing 5% IgG may be stored at room temperature for a period of up to 1 year [22,23]. However, they require more storage space and the high infusion volume may become problematic, particularly in patients with concomitant diseases such as heart failure [21]. The optimal storage temperature for currently available 10% IVIG solutions is 5°C, for a maximal storage time of 36 months. These solutions are stable at room temperature for only a few months, making storage more cumbersome. Furthermore, although short-term exposure to room temperature is tolerated by most concentrated IVIG products, their overall shelf-lives are affected by the length and timing of the exposure to increased temperature [24–27].

Privigen showed excellent stability when stored at room temperature over 36 months: aggregate and fragment levels were detectable but well below the maximum specified levels of 6·0% for fragments and 2·0% for aggregates. The discoloration of Privigen measured by optical density complied with the specified limit up to 36 months storage. The qualitative test parameter 'appearance' (clarity, discoloration, opalescence, particulate matter and flake formation) met the acceptance criteria up to 36 months, with the exception of a single 'fail' result concerning discoloration after 36 months in one 100-ml lot, likely caused by oxidation of immunoglobulins (CSL Behring AG, Bern, Switzerland). Since optical density, a complementary and quantitative parameter indicating the rate of discoloration, did not confirm this out-of-specification result, the 'fail' result of the appearance parameter was considered irrelevant for the long-term stability of Privigen at 25°C up to 36 months. Specific antibody titers remained several-fold higher than the specified limits and the effector function of IgG was preserved during storage of Privigen for 36 months at room temperature.

Idiotype-anti-idiotype dimers bind to each other via antigen-binding regions, effectively recognizing each other as antigens. Because of the nature of their interactions, the relative levels of monomers and dimers equilibrate over a period of a few months and the levels remain largely constant thereafter [28]. Results from our study are in line with this finding: the equilibrium shifted mostly in the first 3–6 months and remained relatively constant for the remainder of the study duration. At room temperature, the dimer levels remained below 8·0% in all lots.

In accordance with the current ICH guidelines on stability testing of new drug substances and products (Q1A(R2) [18] and Q1B [29]), studies at 37°C and at 5°C under light exposure were performed in order to accelerate the degradation of Privigen constituents. As expected, it was possible to exceed the stability threshold of the formulation under further increased physical stressors (data not shown), although product stability was maintained for 6 months at 37°C. This demonstrates the need to respect the storage and handling conditions as described in Methods: Stability assessment. Furthermore, long-term data at 5°C show that Privigen exhibits good stability and low dimer levels under refrigerated conditions (data not shown).

The Privigen lots included in our stability studies were manufactured using the fractionation procedure according to Kistler and Nitschmann [15]. A comparable stability was demonstrated with lots manufactured by using the Cohn method for fractionation (data not shown) [16]. Furthermore, it was demonstrated that the different fill sizes (50 ml and 100 ml) do not show differences in stability of Privigen.

In summary, we show here that the unique formulation of Privigen with L-proline at pH 4·8 allows long-term stability and prevents dimer formation at room temperature. The optimized pH, use of L-proline as stabilizer and the high

purity of Privigen all contribute to its superior stability. Privigen is the first concentrated IVIG solution that can be stored for 36 months at room temperature, in contrast to other currently available IVIG products. This increases the ease of storage and reduces the preparation time for infusions, making Privigen a convenient IVIG for clinical use.

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(54) **PROCESS FOR PRODUCING IMMUNOGLOBULINS FOR INTRAVENOUS ADMINISTRATION AND OTHER IMMUNOGLOBULIN PRODUCTS**

VERFAHREN ZUR HERSTELLUNG VON IMMUNOGLOBULINEN ZUR INTRAVENÖSEN VERABREICHUNG UND ANDEREN IMMUNOGLOBULIN-PRODUKTEN

PROCEDE DE PRODUCTION D'IMMUNOGLOBULINES DESTINEES A UNE ADMINISTRATION INTRAVEINEUSE ET D'AUTRES PRODUITS D'IMMUNOGLOBULINES

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EP 1 084 147 B1

Description

FIELD OF THE INVENTION

[0001] The present invention relates to a process for purifying immunoglobulin G (IgG) from a crude plasma protein fraction. The invention also relates to an immunoglobulin product and to the use of such an immunoglobulin product for medical purposes.

BACKGROUND OF THE INVENTION

[0002] Human normal immunoglobulin (HNI) for use in the prevention and treatment of a number of infectious diseases was introduced in the late 1940's. HNI prepared by the cold ethanol fractionation method according to Cohn & Oncley (Cohn E., et al., (1946), J Am Chem Soc, 68, 459-475), (Oncley et al., (1949), J Am Chem Soc, 71, 541-550) and subsequently also by the modification made by Kistler and Nitschmann (Kistler P and Nitschmann HS, (1952), Vox Sang, 7, 414-424) proved to be both efficient and safe against the transmission of virus infection when administered subcutaneously or intramuscularly.

[0003] Congenital or acquired total or partial lack of immunoglobulin (primary and secondary immunodeficiency syndrome, respectively) manifests itself through frequent ordinary and serious infections, especially of a bacterial nature. The prevention of such infections was previously achieved by repeated intramuscular or subcutaneous injections of large amounts of HNI for up to several times a week as a life-lasting treatment, which is very painful when the medicament is given intramuscularly.

[0004] In the early sixties, administration of HNI by the intravenous route was therefore attempted. Trials showed that about 5% of healthy volunteers and about 95% of patients with an immunoglobulin deficiency developed immediate adverse effects varying from dyspnoea to circulatory shock and being of such serious nature that the intravenous administration of HNI had to be abandoned.

[0005] The reason for the adverse effects mentioned above turned out to be aggregates of immunoglobulins which, among other effects, strongly activated the complement system. This was in particular seen in patients lacking immunoglobulins. Especially serious adverse effects of an anaphylactic nature could be seen in patients who developed antibodies to IgA. Consequently, methods of avoiding aggregate formation and/or eliminating these aggregates during the preparation process were developed, and some twenty years ago the first generation of an immunoglobulin for intravenous administration (IVIG) was tested and found suitable.

[0006] The original purpose of an IVIG was to alleviate infectious episodes in patients with a congenital or acquired total or partial lack of immunoglobulins and to eliminate discomfort in connection with the administration of HNI. Another advantage of IVIG is that large doses of immunoglobulin can be given within a short time, and by this it is possible to obtain sufficiently high blood concentrations very quickly. Especially when treating serious bacterial infections it is of importance to establish high concentrations at sites of infections quickly.

[0007] In recent years, IVIG has furthermore proved to be efficient in other serious diseases, the treatment of which can otherwise be difficult, e.g. haemorrhages caused by the disappearance of the blood platelets on an immunological basis, idiopathic thrombocytopenic purpura (ITP), in some rare diseases such as Kawasaki's syndrome and a number of autoimmune diseases such as polyradiculitis (Guillain Barré's syndrome). Other diseases the treatment of which has been difficult to the present day are currently being subjected to clinical trials with IVIG. The mechanism of action in these diseases has only partly been clarified. The effect is supposed to be related to so-called immunomodulating properties of IgG, e.g. a blockage of Fcγ-receptors on phagocytic cells, increased metabolism of IgG, downregulation of the production of cytokines, and interference with a supposed network of idiotypes/anti-idiotypes, especially relevant for the neutralization of autoimmune reactivity.

[0008] The first generation of IVIG was prepared by pepsin cleavage of the starting material (Cohn fraction II), the purpose of the cleavage being removal of immunoglobulin aggregates. No column chromatography steps were included in the process. The product had to be freeze-dried in order to remain stable for a reasonable period of time and was dissolved immediately prior to use.

[0009] The starting material for the IVIG was HNI which had proved to be safe with respect to the transmission of viruses when used for intramuscular injection. Hence, IVIG was considered to be just as safe. After several years of clinical use, however, IVIG products from some manufacturers were surprisingly shown to cause transfer of hepatitis C virus infection.

[0010] Studies to elucidate the fate of viruses during the production of HNI showed that the removal of virus in the fractionation process from plasma to HNI is modest. The safety of HNI for intramuscular use is likely to be due to the fact that it contains protective immunoglobulins. In combination with the modest volume injected and the intramuscular route of administration, these protective immunoglobulins can neutralize and render common viruses in plasma non-infectious. Especially when large doses of immunoglobulin are given intravenously, virus infections may occur as dem-

onstrated in the early 1990's. Therefore, it was recognized that the production processes should comprise one or more well-defined virus-inactivation and/or removal steps.

[0011] A second generation of IVIG based on uncleaved and unmodified immunoglobulin molecules with low anti-complementary activity and higher stability was introduced in the mid-eighties, but still in the form of a freeze-dried product. This IVIG was purified by several chromatography steps. Products of that kind presently dominate the market for IVIG. The first and second generations of IVIG thus appear as freeze-dried powders which are dissolved immediately prior to use.

[0012] Dissolution of freeze-dried IVIG is slow (up to 30 minutes for one vial). Several portions often have to be dissolved for one patient. As it is of high priority for the users to have an IVIG in a solution ready for use, liquid products have been introduced on the market. More importantly, there is still a need for improvement of the production process in order to obtain a highly purified, stable and fully native IVIG preparation with higher clinical efficacy and less adverse drug reactions. A further developed and improved process for purifying IgG from crude plasma or a plasma protein fraction for a virus-safe, liquid IVIG product is thus needed. Finally, the process should be designed in such a way that it can be used in a large scale production.

[0013] The purification process described in the present application leads to a liquid immunoglobulin product for intravenous administration which can be characterized as a highly purified, fully native, biologically active, double virus-inactivated, and stable new generation of IVIG, which does not contain any detergent, polyethylene glycol (PEG) or albumin as a stabilizer.

[0014] DE 4118912C1 discloses a process for purification of monoclonal IgG molecules obtained from hybridoma cells. This process does not comprise the steps (b) or (d) as described in the present invention and the IgG product does not contain polyclonal IgG of all subclasses IgG_1, IgG_2, IgG_3, and IgG_4, suitable for the treatment of e.g. immune deficiencies and autoimmune diseases as the IgG product of the present invention does.

SUMMARY OF THE INVENTION

[0015] The present invention relates to an improved purification procedure and an improved liquid immunoglobulin product which, *inter alia*, can be administered intravenously.

[0016] An immunoglobulin product obtained by the method of the present invention could be called a third generation IVIG. The process is characterized by the following conditions for fractionation: pepsin cleavage is avoided, aggregates and particles are removed by precipitation (a process step validated to function as a virus removal step), further purification is achieved by column chromatographic ion exchange methods, S/D treatment is introduced as a virus-inactivating step, and the preparation is formulated as a liquid product.

[0017] Due to the improved purity of the immunoglobulin product obtainable by the process of the invention as compared to the prior art products, the addition of stabilizers such as a non-ionic detergent, PEG or albumin is not necessary in order to avoid aggregation of IgG during storage of the IVIG as a liquid product. The product obtainable by the process of the invention has a higher quality than the prior art products and provides improved clinical effects, and unwanted adverse effects are virtually absent.

DETAILED DISCLOSURE OF THE INVENTION

[0018] The present invention relates to a process for purifying immunoglobulin G (IgG), from crude immunoglobulin-containing plasma protein fraction, which process comprises the steps of:

(a) preparing an aqueous suspension of the crude immunoglobulin-containing plasma protein fraction;

(b) adding a water soluble, substantially non-denaturating protein precipitant to said suspension of step (a) in an amount sufficient to cause precipitation of a high proportion of non-immunoglobulin G proteins, aggregated immunoglobulins and particles including potentially infectious particles such as virus particles, without causing substantial precipitation of monomeric immunoglobulin G, thereby forming a mixture of a solid precipitate and a liquid supernatant;

(c) recovering a clarified immunoglobulin G-containing supernatant from the mixture of step (b);

(d) applying the clarified immunoglobulin G-containing supernatant of step (c) to an anion exchange resin and subsequently a cation exchange resin; wherein the anion exchange resin and the cation exchange resin are connected in series and wherein the buffer used for the anion exchange chromatography and the cation exchange chromatography is the same buffer, the pH of said buffer is below 6.0

(e) washing out protein contaminants and the protein precipitant from the cation exchange resin of step (d) with a buffer having a pH and ionic strength sufficient to remove the contaminants from the resin without causing substantial elution of immunoglobulin G;

(f) eluting immunoglobulin G from the cation exchange resin of step (e) with a substantially non-denaturating buffer having a pH value and ionic strength sufficient to cause efficient elution of the immunoglobulin G, thereby recovering an immunoglobulin G-containing eluate;

(g) performing a dia/ultrafiltration on the immunoglobulin G-containing eluate of step (f) to concentrate and/or dialyse the eluate and optionally adding a stabilizing agent;

(h) adding a virucidal amount of virus-inactivating agent to the immunoglobulin G-containing dia/ultrafiltrated and optionally stabilized fraction of step (g) resulting in a substantially virus-safe immunoglobulin G-containing solution;

(i) applying the immunoglobulin G-containing solution of step (h) to an anion exchange resin and subsequently to a cation exchange resin;

(j) washing the cation exchange resin of step (i) with a buffer having a pH and ionic strength sufficient to wash out the protein contaminants and the virus-inactivating agent from the resin without causing substantial elution of immunoglobulin G;

(k) eluting immunoglobulin G from the cation exchange resin of step (j) with a substantially non-denaturating buffer having a pH and ionic strength sufficient to cause efficient elution of the immunoglobulin G, thereby recovering an immunoglobulin G-containing eluate; and

(l) subjecting the immunoglobulin G-containing eluate of step (k) to dia/ultrafiltration to lower the ionic strength and concentrate the immunoglobulin G of the solution, and adjusting the osmolality by adding a saccharide.

[0019] The invention further relates to an immunoglobulin product obtainable by the process, and the use of said product for the preparation of a medicament for the treatment of a mammal with PID (Primary Immune Deficiency), SID (Secondary Immune Deficiency), ITP (Idiopatic Thrombocytopenic Purpura), polyradiculitis, peripheral polyneuropathies, Kawasaki's disease, polymyosilis, severe chronic autoimmune diseases, Chronic Inflammatory demyelinating polyneuropathy (CIDP), multifocal motoric neuropathy, multiple sclerosis, Myasthenia Gravis, Eaton-Lambert's syndrome, Opticus Neuritis, epilepsy, Abortus habitualis, primary antiphospholipid syndrome, Rheumatoid arthritis, systemic lupus erythematosus, systemic scleroderma, vasculitis, Wegner's granulomatosis, Sjögren's syndrome, juvenile rheumatoid arthritis, Autoimmune neutropenia, autoimmune haemolytic anaemia, neutropenia, Crohn's disease, colitic ulcerous, coeliac disease, Asthma, septic shock syndrome, chronic fatigue syndrome, psoriasis, toxic shock syndrome, diabetes, sinuitis, dilated cardiomyopathy, endocarditis, atherosclerosis, and adults with AIDS and bacterial infections.

[0020] The starting material of the present purification process is an immunoglobulin-containing crude plasma protein fraction. The starting material for the purification process can be normal human plasma or may originate from donors with high titers of specific antibodies, e.g. hyperimmune plasma. In the present specification, the term "immunoglobulin-containing plasma fraction" is to encompass all possible starting materials for the present process, e.g. cryoprecipitate-free plasma or cryoprecipitate-free plasma from which various plasma proteins, such as Factor IX and Antithrombin, have been removed, different Cohn fractions, and fractions obtained through precipitation procedures by PEG (Poison et al., (1964), Biochem Biophys Acta, 82, 463-475; Poison and Ruiz-Bravo, (1972) Vox Sang, 23,107-118) or by ammonium sulphate. In a preferred embodiment, the plasma protein fraction is Cohn fractions II and III, but Cohn fraction II, or Cohn fractions I, II and III can be used as well. The different Cohn fractions are preferably prepared from plasma by a standard Cohn-fractionation method essentially as modified by Kistler-Nitschmann. In addition to immunoglobulins, the Cohn fractions contain e.g. fibrinogen, α-globulins and β-globulins, including various lipoproteins, which should preferably be removed during the subsequent purification process: Filter aid may or may not be present depending on the isolation method used to obtain the Cohn fractions (i.e. centrifugation or filtration).

[0021] The first step of the process according to the invention involves preparing an aqueous suspension of an immunoglobulin-containing plasma protein fraction, wherein the IgG concentration in the suspension is sufficiently high so that, during the following precipitation step, a major proportion of the non-IgG-proteins; especially those of higher molecular weight; the aggregated immunoglobulins and other aggregated proteins as well as potentially infectious particles precipitate without substantial precipitation of monomeric IgG. This is generally achieved if the concentration of the IgG in the buffered and filtered suspension is at least about 4 g/l before the addition of the precipitant. It should be taken into consideration that the influence of the protein concentration as well as pH and temperature of the sus-

pension on the precipitation depends on the precipitant chosen.

[0022] It is preferred that the plasma protein fraction is suspended in water and/or buffer at a substantially non-denaturating temperature and pH. The term "substantially non-denaturating" implies that the condition to which the term refers does not cause substantial irreversible loss of functional activity of the IgG molecules, e.g. loss of antigen binding activity and/or loss of biological Fc-function (see Example 2).

[0023] Advantageously, the plasma protein fraction is suspended in water acidified with at least one non-denaturating buffer system at volumes of from 6 to 9, preferably from 7 to 8, times that of the plasma protein fraction. The pH of the immunoglobulin-containing suspension is preferably maintained at a pH below 6, such as within the range of 4.0-6.0, preferably 5.1-5.7, most preferably about 5.4, in order to ensure optimal solubility of the immunoglobulin and to ensure optimal effect of the subsequent PEG precipitation step. Any suitable acidic buffer can be used, but the buffer system preferably contains at least one of the following buffers and acids: sodium phosphate, sodium acetate, acetic acid, HCl. Persons skilled in the art will appreciate that numerous other buffers can be used.

[0024] The immunoglobulin suspension is preferably maintained at a cold temperature, *inter alia* in order to prevent substantial protein denaturation and to minimize protease activity. The immunoglobulin suspension and water as well as the buffer system added preferably have the same temperature within the range of 0-12°C, preferably 0-8°C, most preferably 1-4°C.

[0025] The suspension of an ethanol precipitated paste contains relatively large amounts of aggregated protein material. Optionally, the immunoglobulin-containing suspension is filtered in order to remove e.g. large aggregates, filter aid, if present, and residual non-dissolved paste. The filtration is preferably performed by means of depth filters, e.g. C150 AF, AF 2000 or AF 1000 (Schenk), 30LA (Cuno) or similar filters. The removal of aggregates, filter aid, if present, and residual non-dissolved protein material could also be carried out by centrifugation.

[0026] At least one water-soluble, substantially non-denaturating protein precipitant is added to the immunoglobulin-containing filtered suspension in an amount sufficient to cause precipitation of a high proportion of high molecular weight proteins, lipoproteins, aggregated proteins, among these aggregated immunoglobulins. Other particulate material, such as potentially infectious particles, e.g. virus particles, are also precipitated without causing substantial precipitation of monomeric IgG. The term "infectious particles" in the present context comprises e.g. virus particles (such as hepatitis viruses, HIV1 and HIV2) and bacteria. Substantially non-denaturating, water-soluble protein precipitants are well known in the field of protein purification. Such precipitants are used for protein fractionation, resulting in partial purification of proteins from suspensions. Suitable protein precipitants for use in the process of the present invention include various molecular weight forms of PEG, caprylic acid, and ammonium sulphate. Those skilled in the art will appreciate that several other non-denaturating water soluble precipitants may be used as alternative means for the precipitation. The term "adding a protein precipitant" and variants of that term implies the addition of one or more types of protein precipitation agents.

[0027] A preferred precipitant is the organic agent PEG, particularly PEG within the molecular weight range of 3000-8000 Da, such as PEG 3350, PEG 4000, PEG 5000, and especially PEG 6000 (the numbers of these specific PEG compounds represent their average molecular weight). The advantage of using PEG as a precipitant is that PEG is non-ionic and has protein stabilizing properties, e.g. PEG in low concentration is well known as a stabilizer of IVIG products. The precipitation step also functions as a virus-removal step. PEG concentrates and precipitates the viruses irrespective of the species, size, and surface coating of these.

[0028] A given amount of protein precipitant is added to the filtrated suspension to precipitate the majority of high molecular weight and aggregated proteins and particles, without a substantial precipitation of monomeric IgG, forming a clear supernatant solution. The protein precipitant may be added as a solid powder or a concentrated solution.

[0029] For PEG as precipitant a general rule applies that the higher the molecular weight of the compound, the lower the concentration of PEG is needed to cause protein to precipitate. When PEG 3350, PEG 4000 or preferably PEG 6000 is used, the concentration of the precipitant in the filtrated suspension is advantageously within the range of 3-15% by weight, such as 4-10% (such as about 5%, 6%, 7%, 8%, 9%, 10%), wherein 6% is most preferred. In the precipitation step, the precipitation process is allowed to proceed at least until equilibrium is reached between the solid and the liquid phase, e.g. usually for at least two hours, such as from about 2 hours to about 12 hours, preferably about 4 hours. Throughout the precipitation the suspension is preferably maintained at a low temperature (e.g. less than about 12°C, such as less than about 10°C, preferably between 2°C and 8°C). The most suitable temperature depends on the identity of the protein precipitant.

[0030] After completion of the protein precipitation, a clarified supernatant containing IgG almost exclusively in a monomeric form is recovered from the mixture of solid precipitate and liquid supernatant resulting from the precipitation. The recovery can be performed by conventional techniques for separating liquid from solid phase, such as centrifugation and/or filtration. Preferably, a flow-through centrifuge (e.g. Westfalia) with 1000-5000 g force is used.

[0031] Optionally, the recovered, clarified, IgG-containing supernatant is depth filtered to remove larger particles and aggregates. This is optionally followed by sterile filtration performed by use of a conventional sterilization filter (such as a 0.22 µm filter from Millipore or Sartorius), which eliminates e.g. bacteria from the solution.

[0032] The clarified and optionally filtrated IgG-containing supernatant is subjected to at least one step, such as two steps, but optionally more steps of anion and cation exchange chromatography in order to remove a substantial proportion of the remaining non-IgG contaminants, e.g. IgA, albumin as well as aggregates. In a preferred embodiment, the clarified and optionally filtrated IgG-containing supernatant is applied to an anion exchange resin and subsequently a cation exchange resin packed in two columns of appropriate dimensions.

[0033] When performing the ion exchange chromatography steps for the purification of IgG, it is preferred that the conditions, e.g. the pH and ionic strength, are chosen in such a way that a major portion of the contaminants (e.g. non-IgG proteins such as IgA, transferrin, albumin, and aggregates) in the applied solution binds to the anion exchange resin, whereas substantially no IgG adsorbs to the anion exchange resin. With respect to the subsequent cation exchange chromatography, the preferred conditions chosen result in binding of substantially all of the IgG molecules present in the solution applied to the cation exchange resin. Protein contaminants not adsorbed to the anion exchange resin and the precipitation agent are removed in the subsequent washing of the cation exchange resin.

[0034] In the present process, the anion exchange resin and the cation exchange resin are connected in series. In the present context, the term "connected in series", when used in connection with the ion exchange resins, means that the proteins passing through the anion exchange resin are loaded directly onto the cation exchange resin with no change of buffer or other conditions.

[0035] Several reasons make it advantageous that the anion exchange and cation exchange chromatography is carried out in one step using two serially connected chromatography columns, instead of two independent chromatography steps, e.g. with different buffer compositions. The use of two serially connected chromatography columns makes the operation more practical, e.g. there is no need for an intermediary step of collecting the IgG-containing fraction between the two ion exchange chromatographic methods, for possibly adjusting pH and ionic strength. In addition the buffer flow is applied to both of the columns at the same time, and the two columns are equilibrated with the same buffer. However, it is contemplated that it is also possible to perform the chromatography step in two steps, i.e. the anion exchange resin and cation exchange resin are not connected in series. Performing the chromatography in two steps would though, as mentioned above, be more laborious compared to keeping the ion exchange resins connected in series.

[0036] It is presently contemplated that the high degree of purity, the high content of IgG monomers and dimers and the low content of IgA in the IVIG product of the invention are partly due to the use of two serially connected chromatography columns.

[0037] As will be known by the person skilled in the art, ion exchangers may be based on various materials with respect to the matrix as well as to the attached charged groups. For example, the following matrices may be used, in which the materials mentioned may be more or less crosslinked: agarose based (such as Sepharose CL-6B®, Sepharose Fast Flow® and Sepharose High Performance®), cellulose based (such as DEAE Sephacel®), dextran based (such as Sephadex®), silica based and synthetic polymer based. For the anion exchange resin, the charged groups which are covalently attached to the matrix may e g. be diethylaminoethyl (DEAE), quaternary aminoethyl (QAE), and/or quaternary ammonium (Q). For the cation exchange resin, the charged groups which are covalently attached to the matrix may e.g. be carboxymethyl (CM), sulphopropyl (SP) and/or methyl sulphonate (S). In a preferred embodiment of the present process, the anion exchange resin employed is DEAE Sepharose Fast Flow®, but other anion exchangers can be used. A preferred cation exchange resin is CM Sepharose Fast Flow®, but other cation exchangers can be used.

[0038] The appropriate volume of resin used when packed into an ion exchange chromatography column is reflected by the dimensions of the column, i.e. the diameter of the column and the height of the resin, and varies depending on e g. the amount of IgG in the applied solution and the binding capacity of the resin used.

[0039] Before performing an ion exchange chromatography, the ion exchange resin is preferably equilibrated with a buffer which allows the resin to bind its counterions. Preferably, the anion and cation exchange resins are equilibrated with the same buffer, as this facilitates the process since then only one buffer has to be made and used.

[0040] If, for instance, the chosen anion exchange resin is DEAE Sepharose FF® and the cation exchange resin CM Sepharose FF® and the columns are connected in series, then the columns are advantageously both equilibrated with a non-denaturating acidic buffer having about the same pH and ionic strength as the IgG solution to be loaded. Any of a variety of buffers are suitable for the equilibration of the ion exchange columns, e.g. sodium acetate, sodium phosphate, tris(hydroxymethyl)amino-methane. Persons skilled in the art will appreciate that numerous other buffers may be used for the equilibration as long as the pH and conductivity are about the same as for the applied IgG solution. A preferred buffer for the equilibration of the anion exchange column and cation exchange column when connected in series is a sodium acetate buffer having a sodium acetate concentration within the range of 5-25 mM, such as within the range of 10-20 mM, preferably about 15 mM. It is preferred that the pH of the sodium acetate buffer used for equilibration is within the range of 5.0 to 6.0, such as within the range of 5.4-5.9, preferably about 5.7. The conductivity is within the range of 1.0-1.4 mS/cm, preferably about 1.2 mS/cm. Suitable acetate buffers may be prepared from sodium acetate trihydrate and glacial acetic acid.

[0041] Prior to loading the clarified and optionally filtrated IgG-containing supernatant onto the ion exchange columns, the buffer concentration and pH of said supernatant are preferably adjusted, if necessary, to values substantially equivalent to the concentration and the pH of the employed equilibration buffer.

[0042] After loading the IgG-containing supernatant onto the columns in series, the columns are preferably washed (the initial washing) with one column volume of a washing buffer in order to ensure that the IgG-containing solution is quantitatively transferred from the anion exchange column to the cation exchange column. Subsequently, the anion exchange and the cation exchange columns are disconnected, and the cation exchange column is washed in order to remove protein contaminants from the resin with a buffer having a pH and ionic strength sufficient to elute substantially all of the contaminants from the cation exchange resin without causing substantial elution of IgG.

[0043] The initial washing is advantageously performed by using the equilibration buffer, even though other buffers with a similar concentration and pH-value may be used for the washing. It is preferred that an acetate buffer is used for washing out contaminants from the cation exchange resin. The pH of the buffer could be from 5.0 to 6.0, such as within the range of 5.2-5.8, such as about 5.4.

[0044] The elution of the IgG from the cation exchange resin is performed with a substantially non-denaturating buffer having a pH and ionic strength sufficient to cause efficient elution of the IgG, thereby recovering an IgG-containing eluate. In this context, efficient elution means that at least 75%, such as at least 80%, e.g. at least 85%, of the IgG proteins loaded onto the anion and cation exchange resins in series are eluted from the cation exchange resin. The elution is advantageously carried out as a gradient elution step. In the process of the present invention, the preferred buffer used is sodium acetate having a pH within the range of 5.0-6.0, such as 5.2-5.8, preferably about 5.4, and a concentration within the range of 5-40 mM, such as within the range of 10-25 mM, preferably about 15 mM.

[0045] It is preferred that the salt concentration of the eluting buffer is sufficiently high to displace the IgG from the resin. However, it is contemplated that an increase in pH and a lower salt concentration can be used to elute the IgG from the resin. In a preferred embodiment of the present process, the elution is conducted as a continuous salt gradient elution with sodium chloride concentrations within the range of 50-500 mM, preferably from about 125 mM to about 350 mM sodium chloride.

[0046] The elution can also be performed by step gradient elution. It is contemplated that the elution could also be performed as a constant salt elution, in which the elution buffer applied to the cation exchange column has only one single salt concentration in contrast to the gradient elution. If a constant salt elution is performed, the concentration of salt may advantageously be within the range of from about 350 mM to about 500 mM sodium chloride. The advantage of the gradient elution compared to the constant salt elution is that the elution is more effective with a salt gradient, but another advantage is that the eluate has a lower ionic strength which is advantageous because a high ionic strength is critical to the stability of IgG. The elution buffer may further comprise a protein stabilizing agent such as those mentioned below. Various other suitable buffer systems may be used for eluting the IgG, as will be appreciated by those skilled in the art. Preferably, at least one protein stabilizing agent is applied to the IgG fraction immediately after or during the elution. Protein stabilizing agents are known to those skilled in the art and include e.g. different sugar alcohols and saccharides (such as sorbitol, mannose, glucose, trehalose, maltose), proteins (such as albumin), amino acids (such as lysine, glycine) and organic agents (such as PEG). Advantageously, the intermediary stabilizer chosen may be one that can be removed from the IgG-containing solution in the subsequent steps.

[0047] The suitable concentration of the protein stabilizing agent in the IgG-containing solution depends on the specific agent employed. In one preferred embodiment, the stabilizing agent is sorbitol, preferably at a final concentration within the range of 2-15% (w/v) sorbitol, e.g. about 2.5%.

[0048] Subsequent to elution from the cation exchange column, the eluate is preferably desalinated (i.e. dialysed) and advantageously concentrated. The change of buffer and the concentration of IgG can be performed by a combined dia/ultrafiltration process. The term "dia/ultrafiltration" means that the dialysis and concentration by diafiltration and ultrafiltration, respectively, are performed in one step. It is contemplated that the diafiltration and ultrafiltration may be performed as two separate steps. However, in order to prevent unnecessary loss of the product, it is presently preferred to perform the dialysis and concentration by the methods of diafiltration and ultrafiltration in one step.

[0049] The membranes employed for the dia/ultrafiltration advantageously have a nominal weight cutoff within the range of 10,000-100,000 Da. A preferred membrane type for the present process is a polysulfone membrane with a nominal weight cutoff of 30,000 Da, obtained from Millipore. Other ultrafiltration membranes of comparable material and porosity may be employed.

[0050] The extent of concentration may vary considerably. The solution is concentrated from about 10 g/l IgG to about 100 g/l, preferably to about 50 g/l. Following the concentration, the IgG concentrate is advantageously dialysed against a buffer with low ionic strength. Besides removing salt ions, this step also removes contaminants of low molecular weight from the solution and provides a means for buffer exchange for the next purification step. A preferred buffer for the diafiltration is 15 mM sodium acetate, pH 5.4 containing 2.5% (w/v) sorbitol. The exchange of buffer is continued until the conductivity of the ultrafiltrated solution is reduced to a value less than about 1.5 mS/cm, preferably less than about 1.3 mS/cm. During the dia/ultrafiltration, the pH is preferably kept within the range of 4.0-6.0, preferably

5.1-5.7, most preferably at about 5.4.

[0051] After dia/ultrafiltration, the concentration of the protein stabilizing agent is advantageously adjusted in the solution, if necessary, to the final optimal concentration characteristic for the specific protein stabilizing agent employed. If sorbitol is used, the sorbitol concentration is preferably adjusted to about 10% by weight.

[0052] It is preferred that the stabilized solution is filtered with a filter with a pore diameter within the range of 0.2-1.0 µm, preferably about 0.45 µm, in order to remove aggregates before the next step. At this stage the IgG-containing solution appears as a clear solution of an appropriate volume with a high stability as a combined result of the high purity, the low ionic strength, the acidic pH, the relatively high concentration of IgG and the stabilizer added.

[0053] In the production process of the IVIG product, at least two defined and validated virus removal and inactivation steps are presently incorporated, these steps preferably being precipitation with PEG as a general virus-removal step and an S/D treatment as a virus-inactivating step towards lipid enveloped viruses. Besides the stringent requirements to virus safety of the starting material, according to international guidelines, and the well known virus reducing capacity of a multistep purification process, the incorporation of two independent virus reduction steps being active against both enveloped and non-enveloped viruses, the medicament of the present invention is substantially virus-safe.

[0054] Infectious lipid enveloped viruses that may still be present in the IgG-containing solution are preferably inactivated at this stage of the process by addition of a virucidal amount of virus-inactivating agent to the IgG-containing solution. A "virucidal amount" of virus-inactivating agent is intended to denote an amount giving rise to a solution in which the virus particles are rendered substantially non-infectious and by this a "virus-safe IgG-containing solution" as defined in the art. Such "virucidal amount" will depend on the virus-inactivating agent employed as well as the conditions such as incubation time, pH, temperature, content of lipids, and protein concentration.

[0055] The term "virus-inactivating agent" is intended to denote such an agent or a method which can be used in order to inactivate lipid enveloped viruses as well as non-lipid enveloped viruses. The term "virus-inactivating agent" is to be understood as encompassing both a combination of such agents and/or methods, whenever that is appropriate, as well as only one type of such agent or method.

[0056] Preferred virus-inactivating agents are detergents and/or solvents, most preferably detergent-solvent mixtures. It is to be understood that the virus inactivating agent is optionally a mixture of one or more detergents with one or more solvents. Solvent/detergent (S/D) treatment is a widely used step for inactivating lipid enveloped viruses (e. g. HIV1 and HIV2, hepatitis type C and non A-B-C, HTLV 1 and 2, the herpes virus family, including CMV and Epstein Barr virus) in blood products. A wide variety of detergents and solvents can be used for virus inactivation. The detergent may be selected from the group consisting of non-ionic and ionic detergents and is selected to be substantially non-denaturating. Preferably, a non-ionic detergent is used as it facilitates the subsequent elimination of the detergent from the IgG preparation by the subsequent step. Suitable detergents are described, e.g. by Shanbrom et al., in US Patent 4,314,997, and US Patent 4,315,919. Preferred detergents are those sold under the trademarks Triton X-100 and Tween 80. Preferred solvents for use in virus-inactivating agents are di- or trialkylphosphates as described e.g. by Neurath and Horowitz in US Patent 4,764,369. A preferred solvent is tri(n-butyl)phosphate (TNBP). An especially preferred virus-inactivating agent for the practice of the present invention is a mixture of TNBP and Tween 80, but, alternatively, other combinations can be used. The preferred mixture is added in such a volume that the concentration of TNBP in the IgG-containing solution is within the range of 0.2-0.6% by weight, preferably at a concentration of about 0.3% by weight. The concentration of Tween 80 in the IgG-containing solution is within the range of 0.8-1.5% by weight, preferably at a concentration of about 1% by weight.

[0057] The virus-inactivation step is conducted under conditions inactivating enveloped viruses resulting in a substantially virus-safe IgG-containing solution. In general, such conditions include a temperature of 4-30°C, such as 19-28°C, 23-27°C, preferably about 25°C, and an incubation time found to be effective by validation studies. Generally, an incubation time of 1-24 hours is sufficient, preferably 4-12 hours, such as about 6 hours, to ensure sufficient virus inactivation. However, the appropriate conditions (temperature and incubation times) depend on the virus-inactivating agent employed, pH, and the protein concentration and lipid content of the solution. It is contemplated that other methods for removal of or inactivating virus can also be employed to produce a virus-safe product, such as the addition of methylene blue with subsequent inactivation by radiation with ultraviolet light or nanofiltration.

[0058] After the solvent/detergent treatment, the solution is advantageously diluted with buffer. Optionally, the substantially virus-safe IgG-containing solution is filtered, preferably through a depth filter as described previously in an earlier step of the present process and/or through a sterile filter.

[0059] After virus-inactivation and preferably filtration, ion exchange chromatography is performed in order to remove the virus-inactivating agent and protein contaminants. This step is preferably performed as already described for the previous ion-exchange chromatography step in the present process, with the exceptions that the volume of the anion exchange resin is about half that of the cation exchange resin and that the washing before elution of IgG is more extensive, at least six column volumes of buffer are used. Additionally, in a preferred embodiment of the invention, the equilibration buffer is sodium acetate with a concentration within the range of about 5-25 mM, preferably 15 mM, and a pH within the range of about 5.0-5.8, preferably 5.4. As mentioned previously, the sodium acetate content and the

pH of the IgG-containing solution are preferably adjusted to the same concentration and pH as the equilibration buffer. Additionally, in a preferred embodiment of the invention, a protein stabilizing agent, preferably maltose, is added to the recovered eluate to a final concentration within the range of 1-5%, preferably about 2.5% by weight.

[0060] The preferred method of eliminating the virus-inactivating agent is by ion exchange chromatography. However, other methods, such as oil extraction and alternative chromatographic methods, are contemplated to be useful. The appropriate method depends on the virus-inactivating agent employed. Removal of solvent/detergent may thus be achieved by binding the IgG to a resin and, subsequently, a thorough washing out of the inactivating agent with buffer. Cation exchange chromatography is a usable method. In a preferred embodiment of the present invention, anion exchange chromatography is also performed in addition to the cation exchange chromatography in order to improve the quality and overall purity of the final product of the present process.

[0061] After the ion exchange chromatography step, the IgG-containing eluate is preferably dialysed and concentrated; hereby the content of remaining smaller protein components is also effectively reduced. Advantageously, this can be performed by dia/ultrafiltration as described previously. The buffer employed for the diafiltration is sodium acetate, preferably at a concentration from about 4 to 10 mM, preferably 7.5 mM, and at a pH within the range from about 4.0 to 6.0, preferably about 5.1-5.7, such as about 5.4. Alternatively, other buffers such as sodium phosphate or acids can be used for the diafiltration. The diafiltration continues until the conductivity is less than or equal to 1 mS/cm. Optionally, the IgG-containing solution is further sterile filtered.

[0062] If desired, the purified IgG-containing solution which is substantially free from the virus-inactivating agent is subjected to further treatments for the purpose of making it suitable for formulation as a liquid product to be used e.g. intravenously, subcutaneously, or intramuscularly.

[0063] From a practical point of view it is preferred that the content of the liquid formulation of the immunoglobulin product is the same for storage as for use. The final concentration of IgG in the product is preferably within the range of 0.25-20% by weight (corresponding to 2.5-200 g of IgG/l), such as about 1-20% by weight, i.e. about 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 12%, 14%, 16%, 18%.

[0064] It is known that a high protein concentration results in a higher stability of IgG. On the other hand, a high IgG concentration means that the maximum infusion rate when administering IgG intravenously to the patient has to be quite low as transfusion problems, due to the high osmotic pressure of the product, have to be avoided. A presently recommended concentration for intravenous administration by European Pharmacopoeia (Ph.Eur.) is 5% (w/v). On the other hand, a quite concentrated product (e.g. 10% or above) is advantageous for intramuscular or subcutaneous injections.

[0065] Although not preferred, it is evident that the products obtainable by the various process steps of the invention can also be used as e.g. freeze-dried products instead of as liquid formulations, although this is less favourable compared to the use of the immunoglobulin products as instant liquid formulations. The latter embodiment will be described in more detail in the following.

[0066] Liquid immunoglobulin products are most stable at an ionic strength markedly lower than that of plasma, i.e. the conductivity is preferably less than 1.0 mS/cm, preferably about 0.8 mS/cm.

[0067] The pH has an impact on the stability of IgG and on the infusion rate. Liquid immunoglobulin products are most stable under acidic conditions, i.e. below the isoelectric point of IgG, pH 6.4-8.5. The closer the pH value is to the physiological pH value (7.1-7.3), the higher infusion rate can be employed. As a consequence of the stability required, the pH of the immunoglobulin product of the invention will preferably be within the range of 5.1-5.7, such as between 5.2 and 5.6, such as about 5.4.

[0068] Furthermore, the immunoglobulin product may comprise protein stabilizing agents as described previously. In addition to sugar alcohols and saccharides (such as sorbitol, mannose, glucose, trehalose, maltose), also proteins (such as albumin), amino acids (such as lysine, glycine) and organic agents (such as PEG and Tween 80) may be used as well as stabilizers. The suitable concentration of the stabilizing agent in the IgG-containing solution depends on the specific agent employed as described previously.

[0069] The purified IgG solution is adjusted if necessary in order to obtain a stable and isotonic solution. The term "isotonic solution" is intended to denote that the solution has the same osmotic pressure as in plasma. As mentioned above, the ionic strength is markedly lower in the immunoglobulin product of the invention as a liquid formulation than in plasma. For that reason it is preferred that mono- or disaccharides are used to increase the osmolality of the solution since this does not affect the ionic strength. In a preferred embodiment of the present invention, maltose is added at a concentration ensuring that the solution is isotonic and, at the same time, maltose functions as an immunoglobulin-stabilizing agent. This is preferably performed by addition of maltose to a final concentration within the range of about 5% to 15% (w/v), preferably 10% (w/v); other saccharides, such as mannose and glucose, can alternatively be used.

[0070] The preferred final conditions for the immunoglobulin product are a compromise between stability and physiologically acceptable conditions with respect to e.g. pH, ionic strength and tonicity. Furthermore, the immunoglobulin product has to comply with the requirements of quality control tests, as specified in Monograph No. 918, Ph. Eur., 1997.

[0071] The main advantages of the product obtainable by the method of the invention are that, when formulated as

a liquid preparation, the product is a combination of a liquid, ready-for-use product which, at the same time, is very stable, highly purified, has a largely normal distribution of IgG subclasses and has an extremely low IgA content as well as a low IgM content, and retained antibody activity and biological activity shown by the Fc function.

[0072] Moreover, it contains essentially no aggregates of immunoglobulins and/or other plasma proteins measured as polymers higher than dimers and has a low anticomplementary activity, and it has a very high content of IgG monomers and dimers. Monomeric IgG constitutes at least 90%, which is considered to be ideal. Due to the high stability it is possible to avoid the addition of other stabilizers, such as albumin, glycine, detergent, or PEG. Finally, the product is virus-safe as the process comprises well-defined and validated virus-reduction steps aimed at removing and/or inactivating both lipid enveloped and non-lipid enveloped viruses.

[0073] The aim of validating a production step as a virus reduction step is to provide evidence that the production process will effectively inactivate/remove viruses which are either known to contaminate the starting materials, or which could conceivably do so. Validation studies involve the deliberate addition of a virus prior to the production steps to be validated and measuring the extent of its removal/inactivation after the production step or steps. GMP restraints prevent the deliberate introduction of any virus into the production facilities. Therefore, the validation should be conducted in a separate laboratory equipped for virological work on a scaled-down version of the production step and performed by staff with virological expertise in conjunction with the production engineers. The amount of virus added to the starting material for the production step which is to be validated should be as high as possible in order to determine the capacity of the production step to inactivate/remove viruses adequately. However, the virus spike should be added such that the composition of the production material is not significantly altered. Preferably the volume of the virus spike will be equal to or less than 10%.

[0074] Quantitative infectivity assays should be performed according to the principles of GLP and may involve plaque formation, detection of other cytopathic effects such as syncytia or foci formation, end point titration (eg., $TCID_{50}$ assays), detection of virus antigen synthesis or other methods. The method should have adequate sensitivity and reproducibility and should be performed with sufficient replicates and controls to ensure adequate statistical accuracy of the results.

[0075] Typically, a process step is challenged with 6 logs of virus, and if a reduction in the order of 4 logs or more is acquired, it is indicative of a clear effect with the particular test virus under investigation. Similarly, a reduction in the order of 4.5 logs, 5 logs, or even 5.5 logs, is indicative of a clear effect with the particular test virus under investigation, and the step can be classified as a validated virus reduction step

[0076] The virus validation studies should be performed with viruses resembling those which may contaminate the product as closely as possible and secondly to represent as wide a range of physico-chemical properties as possible in order to test the ability of the system to eliminate viruses in general.

[0077] The virus validation studies should be performed in accordance with the CPMP Note for Guidance on Virus Validation Studies: The Design, Contribution and Interpretation, of Studies Validating the Inactivation and Removal of Viruses (CPMP/BWP/268/95) and Note for Guidance on Plasma Derived Medicinal Products (CPMP/BWP/269/95).

[0078] The validation studies of the present process are presented in example 5.

[0079] The product of the invention is more than 95% pure, preferably above 98%. The high degree of purity is, *inter alia,* due to the fact that the product of the invention is obtained by at least one, preferably two, optionally serially connected anion-cation exchange chromatography steps. It is noteworthy in this context that it has been possible to obtain a high yield in spite of the number of process steps employed, in production scale of at least 3.5 g of IgG protein per kg of fresh frozen plasma.

[0080] The comparative studies which have been carried out (Example 2) have shown that the immunoglobulin product obtainable by the process of the invention has ideal functional properties, such as prominent antigen binding activities and a high Fc function. The presently preferred medicament developed by the present inventors is a 5% by weight immunoglobulin solution. Stability tests have so far indicated stability at 4°C storage for more than one year, i. e. that the immunoglobulin product is devoid of aggregate formation or fragmentation of immunoglobulins G, loss of the desired biological activity, or increase of undesired activities, e.g. anticomplementary activity and prekallikrein activity as measured *in vitro.*

[0081] Based on the present invention, it is possible to obtain an IgG product that is more than 95%, such as at least 96%, or at least 97%, e.g. at least 98%, preferably at least 99%, more preferably at least 99.5% pure. The IgG product should contain less than 6 mg of IgA/l, such as less than 4 mg of IgA/l, preferably less than 3 mg of IgA/l, more preferably less than 2 mg of IgA/l.

[0082] It should be stressed that other products contain stabilizers in the form of a detergent, PEG, or albumin. In a preferred embodiment, the product of the present invention does not contain any of said stabilizers, instead a well-tolerated saccharide has been chosen.

[0083] The product of the present invention has, as one of its characteristics, a very low content of polymers and aggregates. In a preferred embodiment, the product of the present invention contains less than 1.5% polymers and aggregates, such as less than 1%, e.g. less than 0.5%, or less than 0.25% polymers and aggregates. The content of

IgG monomers and dimers is at least 95%, such as at least 96%, or at least 97%, e.g. at least 98%, preferably at least 98.5%, or 99%. The content of monomeric IgG is at least 90% in the product.

[0084] Trials have shown clinical effect of the product of the present invention comparable to registered IVIG products. The product has been well-tolerated by the patients, and the turnover time of the immunoglobulins in circulation has been determined to be four weeks. In the present trials, the immunomodulating effect of IVIG, SSI has been shown to be convincing (data are presented in example 3).

[0085] The indications for IVIG are primary hypo/agammaglobulinaemia including common variable immunodeficiency, Wiskott-Aldrich syndrome and severe combined immunodeficiency (SCID), secondary hypo/agammaglobulinaemia in patients with chronic lymphatic leukaemia (CLL) and multiple myeloma, children with AIDS and bacterial infections, acute and chronic idiopathic thrombocytopenic purpura (ITP), allogenic bone marrow transplantation (BMT), Kawasaki's disease, and Guillan-Barré's syndrome.

Neurology: Chronic inflammatory demyelinating polyneuropathy (CIDP), multifocal motoric neuropathy, multiple sclerosis, Myasthenia Gravis, Eaton-Lambert's syndrome, Opticus Neuritis, epilepsy.

Gynaecology: Abortus habitualis, primary antiphospholipid syndrome.

Rheumatology: Rheumatoid arthritis, systemic lupus erythematosus, systemic scleroderma, vasculitis, Wegner's granulomatosis, Sjögren's syndrome, juvenile rheumatoid arthritis.

Haematology: Autoimmune neutropenia, autoimmune haemolytic anaemia, neutropenia.

Gastrointestinal: Crohn's disease, colitic ulcerous, coeliac disease. Others: Asthma, septic shock syndrome, chronic fatigue syndrome, psoriasis, toxic shock syndrome, diabetes, sinuitis, dilated cardiomyopathy, endocarditis, atherosclerosis, adults with AIDS and bacterial infections.

[0086] Apart from the mentioned indications for treatment with IVIG products, several severe autoimmune diseases, which commonly respond to cortico-steroid and immunosuppressive therapy, are considered target conditions for therapy with the product of the present invention. Among these are several neurological diseases such as polyradiculitis, and some immune-mediated peripheral polyneuropathies, but also some chronic inflammatory rheumatic and vascular conditions such as systemic vasculitis involving small vessels, polymyositis, and others.

[0087] A different mode of action of the product of the present invention may be the elimination of infectious antigens in chronic infections and an increase of IgG metabolism.

[0088] The invention is further illustrated by the following examples, which are not intended to be limiting.

EXAMPLES

Example 1

PROCESS STEPS IN THE PURIFICATION OF IMMUNOGLOBULIN (with the exception of step 5, all steps are carried out at 5±3°C)

Step 1: Preparation of Cohn fraction II + III paste:

[0089] Cohn fraction II + III paste is prepared from human plasma by the standard Cohn-fractionation method (Cohn E., et al., (1946) J Am Chem Soc, 459-475) essentially as modified by Kistler-Nitschmann (Kistler P and Nitschmann HS, (1952), Vox Sang, 7, 414-424). The ethanol precipitation is initiated after the cryoprecipitate has been removed and, if desired, after adsorption of certain plasma proteins (such as Factor IX and Antithrombin) to e.g. an ion exchange material and/or a Heparin Sepharose® matrix.

[0090] The exact conditions (pH, ethanol concentration, temperature, protein concentration) for obtaining the fraction II-III paste appear from the figure at page 266 in Hams JR (ed), Blood Separation and Plasma Fractionation, Wiley-Liss, New York, 1991. The paste is isolated on a filter press by adding filter aid prior to filtration.

Step 2: Extraction of immunoglobulins from Cohn fraction II + III paste:

[0091] From 140 kg of fraction II + III paste including 30 kg of filter aid (Schenk, Germany) (corresponding to a starting volume of plasma of about 1150 kg), extraction is accomplished by first adding 525 kg of 2.33 mM sodium phosphate/acetate buffer, pH 4.0, with slow stirring for about 1.5 hours, followed by 2 consecutive additions of 350 kg of water for injection (WFI) with stirring for about 1.5 hours after each addition. Finally, about 280 kg of 21.5 mM sodium phosphate/acetate, pH 7.0, are added, thereby adjusting the pH of the suspension to 5.4.

[0092] The suspension is filtered through a depth filter (C-150AF, Schenk, Germany). The filtrate contains, among other proteins, the immunoglobulins.

Step 3: Precipitation of protein aggregates and removal of virus by PEG 6000:

[0093] PEG 6000 (Merck, Germany) is added to the filtrate of step 2 to a final concentration of 6% by weight. After precipitation for 4 hours, the PEG suspension is centrifuged to clarity in a flow-through centrifuge (Westfalia BKA28, Germany) and is depth filtered (50LA and 90LA, Cuno, France) and subsequently sterile filtered through a 0.22 µm filter (Durapore, Millipore, U.S.A.). The filtered PEG supernatant is buffer-adjusted by adding 1 part of a 0.45 M sodium acetate buffer, pH 5.7, to 29 parts of supernatant to reach a pH of 5.7.

Step 4: Purification by serial anion and cation exchange chromatography (I):

[0094] Two chromatography columns are packed with 56 l of DEAE Sepharose FF® (Pharmacia Biotech, Sweden) and 56 l of CM Sepharose FF® (Pharmacia Biotech, Sweden), respectively. The columns are connected in series so that the liquid flow first passes through the DEAE Sepharose resin and, subsequently, through the CM Sepharose resin. The column resins are equilibrated with 15 mM sodium acetate buffer, pH 5.7. Then, the solution from step 3 is applied to the two columns in series.

[0095] During the ion exchange chromatography, most contaminating proteins in the applied solution bind to the DEAE Sepharose resin. Whereas IgG runs through without binding to the DEAE Sepharose resin, IgG binds to the CM Sepharose resin when the solution migrates through it. After application of the solution, and washing with one column volume of equilibration buffer, the DEAE column is disconnected from the CM column. Then the CM column is washed with three column volumes of 15 mM sodium acetate buffer, pH 5.4, then IgG is eluted with a gradient of NaCl from 125 mM to 350 mM NaCl, 15 mM sodium acetate, pH 5.4. The eluted IgG fraction is collected in sorbitol to a final concentration of 2.5% by weight.

Step 5: Solvent/detergent (S/D) treatment of the IgG fraction:

[0096] The eluted IgG fraction is concentrated and desalted by ultra/diafiltration to a concentration of approximately 50 g of IgG/litre. The employed membrane is a polysulfone membrane, nominal weight cutoff of 30 kDa (Millipore). The diafiltration is performed against a buffer of 15 mM sodium acetate, pH 5.4, containing 2.5% by weight of sorbitol and is continued until the conductivity is less than 1.4 mS/cm. The IgG content of the solution is determined spectrophotometrically by measuring at 280 nm (A_{280}). The sorbitol concentration is adjusted to 10% by weight and the solution is filtered through a 0.45 µm filter (Pall Corporation, UK). Tween 80 and TNBP are then added to a final concentration of 1% and 0.3% by weight, respectively, for subsequent S/D treatment. The S/D treatment proceeds for at least 6 hours at 25°C.

Step 6: Removal of S/D by ion exchange chromatography (II):

[0097] Two serially connected columns packed with 28 l of DEAE and 56 l of CM Sepharose FF, respectively, are equilibrated with 15 mM sodium acetate, pH 5.4. The S/D-treated IgG fraction from step 5 is diluted with 5 parts of 15 mM acetate buffer, pH 5.4, filtered through a depth filter (Cuno 90 LA) and subsequently sterile filtered (Sartobran, Sartorius), and applied to the two columns connected in series. The ion exchange chromatography and the subsequent elution of IgG from the CM column are carried out essentially as described in step 4, except that the CM column is extensively washed with 6 column volumes of buffer to remove agents from the S/D treatment. The eluted IgG fraction is collected in maltose (Merck, Germany) to a final concentration of 2.5% by weight.

Step 7: Final concentration and formulation of immunoglobulin for intravenous use:

[0098] The eluted IgG fraction from step 6 is subjected to ultrafiltration and desalting by diafiltration against 7.5 mM sodium acetate containing 2.5 % by weight of maltose, pH 5.4 to a final conductivity of less than 1 mS/cm. The employed membrane is a polysulfone membrane with a 100 kDa nominal weight cutoff allowing proteins with lower molecular weight to be eliminated. The final concentration of IgG is adjusted to 50 g/litre, and the maltose is adjusted to a final concentration of 10% (w/v). The maltose-adjusted final preparation is filtered through a sterile filter (Sartopure GF 2, Sartorius), and filled aseptically.

Example 2

[0099]

RESULTS FROM AN ANALYTICAL STUDY OF A PRODUCT OBTAINED BY THE PRESENT PROCESS, COMPARED TO OTHER IVIG PRODUCTS				
	Gammonativ	Octagam	Gammagard	IVIG, SSI
	lyophilized	liquid	lyophilized	liquid
Purity	45.4%[1]	99.1%	94.6%[1]	99.8%
Albumin	50 mg/ml[2]	small amounts	3 mg/ml[2]	not detectable
Content of monomers+dimers	98.3%[3]	96.8%	97.6%[3]	99.3%
polymers+aggreg	0.8%[3]	1.6%	<0.1%[3]	<0.1%
ACA	26%	30%	34%	32%
PKA	<8.5 IE/ml	<8.5 IE/ml	<8.5 IE/ml	<8.5 IE/ml
Haemagglutinin, 3% solution				
anti-A > 1:2	negative	negative	negative	negative
anti-B > 1:2	negative	negative	negative	negative
Fc function	169%	121%	132%	178%
Subclass distribution				
IgG1	60.0%	61.9%	67.7%	56.6%
IgG2	35.8%	33.1%	27.2%	39.4%
IgG3	3.5%	3.6%	4.4%	2.6%
IgG4	0.7%	1.4%	0.6%	1.5%
IgA	2.96 mg/l	54.7 mg/l	0.85 mg/l	1.36 mg/l
IgM	0.28 mg/l	39.1 mg/l	0.99 mg/l	0.16 mg/l
Tween 80	<20 ppm	<20 ppm	not determined	<20 ppm
TNBP	2.0 ppm	1.5 ppm	1.5 ppm	1.5 ppm
PEG	0.01 mg/ml	0.01 mg/ml	1.6 mg/ml[4]	0.02 mg/ml
pH	6.7	5.7	6.7	5.6
Total protein concentration	97 g/l	45 g/l	50 g/l	51 g/l
Maltose or glucose	20 mg/ml	92 mg/ml	15 mg/ml	88 mg/ml

1: without correction for HSA;

2: declared by producer;

3: corrected for HSA peak;

4: used as a stabilizer

Purity (protein composition)

[0100] Pharmacopoeia purity requirements for an IVIG-preparation is at least 95% IgG, that is not more than 5% non-IgG-contaminating proteins present. Purity is regarded as being of very high importance for several reasons. From a rational point of view, only the protein which carries the desired function should be present, and other contaminating proteins may be potentially harmful, e.g. cause unwanted adverse effects and/or influence the stability of the product.

[0101] Purity can e.g. be analyzed by an electrophoretic technique as described in detail in Ph. Eur., 1997, pages 964-965, where proteins are separated in a cellulose acetate gel. For practical purposes, however, an agarose gel is used. After electrophoresis, the gel is fixed, dried, and stained. Protein bands are finally monitored by scanning. It appears from the table above that the product of the invention is virtually pure (99.8%).

Albumin

[0102] The albumin content was analyzed by crossed immuno-electrophoresis essentially as described by C.B. Laurell (Anal Biochem (1965), 10, 358-361). 5 µl of product was analyzed against anti-human albumin antibodies (DAKO A/S, Denmark, No. A0001 (1/100)). Due to the high purity no albumin was detectable in the analyzed product of the invention.

Content of IgG monomers and dimers

[0103] The content of IgG monomers and dimers can be analyzed by gel permeation chromatography, and monitored from the chromatogram by integration of the areas of the monomer and of the dimer peak, cf. Ph.Eur. The results of the various analyses are listed in the table above from which it appears that the sum of the monomer + dimer areas constitutes 99.3% of the total area of the chromatogram (from this monomeric IgG constitutes 92%) for the product of the invention.

Content of polymers and aggregates

[0104] The presence of polymers and aggregates is known to be the cause of serious adverse effects, often influenza-like symptoms. Because of the very high degree of purity reached by the rather gentle production process, the immunoglobulin product obtainable by the process of the invention is largely free of polymers and aggregates. Polymers can be analyzed by gel permeation chromatography, and any protein peaks with retention times shorter than the retention time for dimeric IgG are considered polymeric as described in Ph.Eur.

[0105] According to Ph.Eur. and other guidelines, the content of protein aggregates should preferably be less than 3%. The product of the present process contains no measurable aggregates and is thus considered to contain less than 0.1% polymers and aggregates.

Anti-complementary activity (ACA) and prekallikrein activator activity (PKA)

[0106] ACA and PKA are measured as described in Ph.Eur.

[0107] ACA should preferably be as low as possible. According to Ph.Eur. the complement consumption should be less than or equal to 50%. The complement consumption of the measured sample of the product of the invention is about 30%, i.e. comparable to that of the other products analyzed. It should be noted that the presence of albumin tends to suppress complement consumption (inventor's observation).

[0108] PKA, if present in substantial amounts, is essential for the hypotensive adverse effect of the product. Therefore, PKA should preferably be as low as possible in an immunoglobulin product. According to Ph.Eur. it should be <35 IU/ml when measured as outlined in Ph.Eur. PKA of the product of invention as well as of the other products analyzed is less than the quantitation level of the method, i.e. below 8.5 IE/ml.

Haemagglutinins

[0109] The IgM fraction of plasma immunoglobulins comprises the haemagglutinins, that is antibodies against blood type A and B antigens. The presence of such antibodies may cause unwanted adverse effects due to a possible haemolytic reaction if the recipient carries blood types A and/or B.

[0110] According to Pharmacopoeia requirements, the content of haemagglutinins must be lower than that causing agglutination of A/B erythrocytes in a dilution 1:64 of the immunoglobulin product. All the products analyzed fulfil this requirement.

Fc-function

[0111] Retained antigen binding activities are essential for the biological functions of the IVIG. This is also true for the immunomodulating activities. On the other hand, a retained Fc-function is essential for the effect of IVIG on various phagocytic cells and activation of the complement system. Fc-function can be demonstrated using various techniques, but an accepted methodology described in Ph.Eur. measures the complement-activating potential of antibodies in the preparation against rubella antigen. Activity is compared to that of a biological reference preparation (BRP, Ph.Eur.) of immunoglobulins set to be 100%. The product complies with the test if the relative activity is more than 60% of the reference preparation. It appears that the Fc-function of the product of the invention is very well preserved, particularly in comparison with the other liquid product analyzed, most likely due to the gentle purification process.

Subclass distribution

[0112] The distribution of IgG subclasses is measured by a standard Mancini immunodiffusion method essentially as described by A. Ingild (Scand J Immunol, (1983), 17, 41). The concentrations are determined by use of a WHO reference serum (67/97). It is required that the subclass distribution should be within the range of normal human plasma with median concentrations in the range of 3.7 -10.2 g IgG1/l serum, 1.1 - 5.9 g IgG2/l serum, 0.15 - 1.3 g IgG3/l serum, and 0.06 - 1.9 g IgG4/l serum (R. Djurup et al. Scand J. Clin Lab Invest 48, 77-83). Thus, the subclass distribution of all the products is acceptable.

IgA-content

[0113] The presence of IgA is known to potentially cause sensibilisation of IgA-deficient recipients. If an IgA-deficient patient receives an IgA-containing immunoglobulin preparation, IgA may be considered as a foreign antigen, and the result may be the induction of antibodies against IgA in the recipient. The next time an IgA-containing preparation is infused to the patient, an anaphylactic reaction may be provoked. It is therefore essential that an immunoglobulin preparation contains as little IgA as possible. IgA in an IVIG product can be monitored using an ELISA-technique, e. g. where a polyclonal anti-IgA is used to capture IgA, and a labelled anti-IgA is used for the detection of bound IgA. Standards are constructed by dilutions of a calibrator (No. X908, DAKO A/S, Denmark) with a declared IgA-content. The product of the process described in Example 1 contains less than 2 mg of IgA/l which is a considerably lower IgA-content than that of the other analyzed liquid product. The physico-chemical similarities between IgG and IgA make it difficult to separate these immunoglobulins during a purification process. However, the two anion/cation exchange chromatography steps in the process reduce the IgA-content to a very low level.

IgM-content

[0114] IgM in an Ig-preparation can be monitored using an ELISA-technique, e.g. where a polyclonal anti-IgM is used to capture IgM, and a labelled anti-IgM is used for detection. Standards are constructed by dilutions of a calibrator (No. X908, DAKO A/S, Denmark) with a declared IgM-content. It appears from the table that the IgM-content of the product of the invention is very low and markedly lower than that of the other liquid product.

Tween 80, TNBP and PEG

[0115] Tween 80, TNBP and PEG are measured by standard procedures. In general, the content of these additives should be as low as possible.

pH

[0116] pH of the analyzed liquid products is acidic, pH 5.6-5.7, whereas the analyzed lyophilized products are neutral after dissolution, with a pH of 6.7.

Total protein concentration

[0117] According to Ph.Eur. the protein concentration should be at least 50 g/l ±10%; all the products fulfil this requirement. The protein concentration is measured by the method of Kjeldahl.

Maltose and glucose stabilizers

[0118] Saccharides are commonly used stabilizers of immunoglobulin products, they have good stabilizing properties and are quickly excreted. The content of maltose, sucrose, and glucose is determined by use of a commercial kit (Boehringer Mannheim, Germany) with maltose as a reference.

[0119] It appears that the two lyophilized products stabilized by albumin and albumin as well as PEG, respectively, also contain a saccharide stabilizer in concentrations from about 15 mg/ml to 20 mg/ml. The product of the invention and the other liquid product are very equally stabilized, i.e. with about 9%, 88 mg/ml and 92 mg/ml, of maltose. By regarding the content of polymers and aggregates as a parameter of stability, the product of the invention has a higher stability than the other liquid product analyzed, although their formulations appear very similar.

Example 3

RESULTS FROM CLINICAL TRIALS

[0120] The clinical studies of the product of the present invention, also referred to as IVIG, SSI, are carried out in accordance with ICH and CPMP/388/95 guidelines.

[0121] Pharmacokinetics, effect and safety have been examined. The clinical trials have so far included four groups of patients: patients with primary immunodeficiency syndrome (15 patients), secondary immunodeficiency syndrome (6 patients), idiopathic thrombocytopenic purpura (15 patients) and patients with chronic inflammatory demyelinating polyneuropathia (5 patients).

[0122] Patients with primary immunodeficiency syndrome or secondary immunodeficiency syndrome were treated with 0.2-0.4 g/kg with intervals of 2-5 weeks. Patients with idiopathic thrombocytopenic purpura were treated with 400 mg/kg per day for five days or with 1000 mg/kg per day for two days.

[0123] For safety measures serum-transaminases, serum-creatinine and virus markers have been determined in all patients. Five patients with idiopathic thrombocytopenic purpura have been followed for virus, kidney and liver safety markes for up to a total of 24 weeks.

Pharmacokinetics

[0124] $T_{½}$ was measured to 30,5 days (median). This is in accordance with results of other IVIG medicaments.

Effect

[0125] For patients with primary and secondary immunodeficiency syndrome, days lost through sickness, hospitalisations, days with antibiotics, days with fever and the number of pneumonias were registered retrospectively for a 6-month period during which the patients had been treated with other registered IVIG medicaments. In the following 6 months during which the patients were treated with Immunoglobulin SSI, liquid, the same parameters were registered.

[0126] The conclusion is that Immunoglobulin SSI, liquid is just as effective as other IVIG compositions for the prophylaxis/prevention of infections in patients with primary and secondary immunodeficiency syndrome.

[0127] In 80% of patients with idiopathic thrombocytopenic purpura, the number of platelets raised from $<30 \times 10^9$/L before the treatment with Immunoglobulin SSI, liquid to $\geq 50 \times 10^9$/L after the treatment. The increase in the platelet count and the duration of the remission in the individual patient were on the same level as after administration of the same dose of other IVIG medicaments, in the cases where comparison was possible. One patient receiving IVIG for the first time was refractory to the test drug. Such a reaction to IVIG is not infrequent, and thus not surprising. Details of the rise of platelets and the duration of the rise are under way.

[0128] The conclusion is that Immunoglobulin SSI, liquid is just as effective as other IVIG medicaments in the treatment of low platelet count in patients with chronic idiopathic thrombogenic purpura.

[0129] According to clinicians, and patients suffering from chronic inflammatory demyelinating polyneuropathia, the IVIG, SSI has shown identical efficacy to the IVIG administrered prior to the trial. IVIG, SSI was tolerated by the patients equally well as other IVIG products were tolerated by the patients.

Safety

[0130] Apart from one severe adverse event, splenectomia assessed by the investigator to have no relation to test drug, only minor adverse events have been registered. These adverse effects were mainly headache fever, and vomiting. So far, there have been no reports on abnormal vital signs during infusions of IVIG, SSI. No viral seroconversions have been registered. There have been no reports on kidney or liver damages or cases of anaphylactic shocks.

[0131] The clinical studies show that Immunoglobulin SSI, liquid is well tolerated. The frequency of side effects, degree and species does not deviate from experiences with other IVIG medicaments.

Example 4

RESULTS FROM STABILITY STUDY FOR IVIG LIQUID

[0132] In order to test if the liquid IVIG product is stable over time, a Real time Real conditions study of stability was conducted. A total of 4 consecutive batches (250 ml of each sample) of the IVIG product were involved in the study and stored at between 2°C - 8°C for at least 12 months. Samples from the four batches were analyzed at time zero, 6 month at storage and 12 months at storage. The results of the study are presented below as means of 4 batches.

	0 months of storage	6 months of storage	12 months of storage
Appearance	Slightly opalescent and colourless	Slightly opalescent and colourless	Slightly opalescent and colourless
Content of monomers+dimers	100%	99.6%	99.5%
polymers+aggreg	<0.1%	<0.1%	<0.1%
ACA	39.7%	38.2%	37.3%
PKA	<8.5 IE/ml	<8.5 IE/ml	<8.5 IE/ml
Fc function	107%	113%	111%
Subclass distribution			
IgG1	59.2%	57.7%	57.1%
IgG2	36.4%	38.1%	38.6%
IgG3	2.7%	2.6%	2.5%
IgG4	1.8%	1.6%	1.7%
pH	5.5	5.5	5.5
Protein composition (% IgG)	99.8%	99.7%	99.1%
Total protein concentration	48.8 g/l	48.3 g/l	49.2 g/l
Osmolality	348 mOsm/kg	347 mOsm/kg	350 mOsm/kg

[0133] All the above mentioned tests were carried out in accordance with Ph.Eur. and as described in Example 2.

[0134] The observation that the content of monomers and dimers is constant over a period of 12 months indicates that polymers are not formed in the sample. The presence of immunoglobulin polymers is known, among others, to be the cause of serious adverse effects, often influenza-like symptoms. Because of the very high stability of the immunoglobulin product obtainable by the process of the invention, the product is largely free of polymers and aggregates even after a long period of storage.

[0135] No increase in ACA is observed over time, although batches expressing rather high ACA deliberately have been included in this stability study. If an increase in ACA was observed, it might indicate that aggregates were being formed during storage. Thus, the constant ACA over time indicates that no aggregates are being formed.

[0136] The results further indicate that no prekallikrein activator activity has developed during storage of the product, as the PKA activity does not increase. It should be noted, however, that the values measured are below the lower quantification level.

[0137] The measure of Fc-function indicates that the presence of intact functional IgG is maintained during storage. Thus, no proteases are present in the samples, as they would have degraded the proteins and thereby lowered the Fc-function. Denaturation of IgG molecules has neither taken place as this would have decreased antigen binding activity.

[0138] As it will be known by the person skilled in the art, there might be difference in the stability of the various subclasses of IgG. As can be seen from the present results, all subclasses are maintained during storage indicating that the product is stable. This is further supported by the finding that the protein composition of IgG in the samples with approximately the same total protein concentration is almost unchanged over time, indicating that there is no overall degradation of IgG. i.e. the product of the present invention is stable and can be stored at least for 12 months at 2-8°C without significant changes of characteristics, and by this efficacy and safety is demonstrated.

Example 5

VALIDATED VIRUS REDUCTION STEPS IN THE PRESENT PROCESS OF IVIG PRODUCT

VIRUS REMOVAL BY A PARTITIONING STEP

[0139] Precipitation of virus present in the immunoglobulin solution by polyethylene glycol Virus validation studies have been performed employing two small non-envelopped viruses, the following virus reductions were obtained:

removal of 6.3 $\log_{10}$ of Hepatitis A Virus (HAV)
removal of 7.2 $\log_{10}$ of Polio Virus

Virus validation studies have been performed employing two enveloped viruses, the following virus reductions were obtained:

removal of 7.6 $\log_{10}$ of HIV
removal of 7.5 $\log_{10}$ of BVDV

VIRUS INACTIVATION BY A S/D TREATMENT STEP

[0140] Treatment of the immunoglobulin solution with 1% Tween 80 + 0.3% TNBP, at 25°C for ≥ 6 hours.
Virus validation studies have been performed employing four enveloped viruses, the following virus reductions were obtained:

inactivation of 7.4 $\log_{10}$ of HIV
inactivation of 5.3 $\log_{10}$ of Sindbis Virus
inactivation of 4.1 $\log_{10}$ of BVDV
inactivation of 5.1 $\log_{10}$ of PRV

[0141] A total of 8 validation studies have been performed on two different steps in the process of the present invention. The PEG precipitation step has been validated as a virus removal step employing four different viruses, two small non-enveloped viruses HAV and Polio virus, and two enveloped viruses HIV and BVDV as model for Hepatitis C Virus. These studies showed that all four viruses were efficiently removed by PEG precipitation. The PEG precipitation step is therefore validated as an efficient virus removal step. The S/D treatment has been validated employing four different enveloped viruses. From the data of the validation studies it appears that the S/D treatment step efficiently inactivated all four viruses. The S/D treatment step is therefore validated as an efficient virus inactivation step. Both virus reduction steps in the MG process, removal by PEG precipitation and inactivation by S/D treatment, have been validated efficiently to remove and inactivate four different viruses each. The cumulative reduction factors of HIV and BVDV in the process are 15 and 11.6, respectively. By this the product of the present process can be regarded as virus safe.

Claims

1. A process for purifying immunoglobulin G (IgG), from a crude immunoglobulin-containing plasma protein fraction, which process comprises the steps of:

(a) preparing an aqueous suspension of the crude immunoglobulin-containing plasma protein fraction;

(b) adding a water soluble, substantially non-denaturating protein precipitant to the said suspension of step (a) in an amount sufficient to cause precipitation of a high proportion of non-immunoglobulin G proteins, aggregated immunoglobulins and particles including potentially infectious particles such as virus particles, without causing substantial precipitation of monomeric immunoglobulin G, thereby forming a mixture of a solid precipitate and a liquid supernatant;

(c) recovering a clarified immunoglobulin G-containing supernatant from the mixture of step (b);

(d) applying the clarified immunoglobulin G-containing supernatant of step (c) to an anion exchange resin and subsequently a cation exchange resin, wherein the anion exchange resin and the cation exchange resin are connected in series and wherein the buffer used for the anion exchange chromatography and the cation exchange chromatography is the same buffer, the pH of said same buffer is below 6.0.

(e) washing out protein contaminants and the protein precipitant from the cation exchange resin of step (d) with a buffer having a pH and ionic strength sufficient to remove the contaminants from the resin without causing substantial elution of immunoglobulin G;

(f) eluting immunoglobulin G from the cation exchange resin of step (e) with a substantially non-denaturating buffer having a pH and ionic strength sufficient to cause efficient elution of the immunoglobulin G, thereby recovering an immunoglobulin G-containing eluate;

(g) performing a dia/ultrafiltration on the immunoglobulin G-containing eluate of step (f) to concentrate and/or

dialyse the eluate, and optionally adding a stabilizing agent;

(h) adding a virucidal amount of virus-inactivating agent to the immunoglobulin G-containing dia/ultrafiltrated and optionally stabilized fraction of step (g) resulting in a substantially virus-safe immunoglobulin G-containing solution;

(i) applying the immunoglobulin G-containing solution of step (h) to an anion exchange resin and subsequently to a cation exchange resin;

(j) washing the cation exchange resin of step (i) with a buffer having a pH and ionic strength sufficient to wash out the protein contaminants and the virus-inactivating agent from the resin without causing substantial elution of immunoglobulin G;

(k) eluting immunoglobulin G from the cation exchange resin of step (j) with a substantially non-denaturating buffer having a pH and ionic strength sufficient to cause efficient elution of the immunoglobulin G, thereby recovering an immunoglobulin G-containing eluate; and

(l) subjecting the immunoglobulin G-containing eluate of step (k) to dia/ultrafiltration to lower the ionic strength and concentrate immunoglobulin G of the solution, and adjusting the osmolality by adding a saccharide.

2. A process according to claim 1, wherein the immunoglobulin G-containing plasma protein fraction is selected from the group consisting of Cohn fraction II; Cohn fractions II and III; and Cohn fractions I, II and III.

3. A process according to claim 1 or 2, wherein the suspension in step (a) is maintained at a temperature from 0°C to 12°C and the suspension in step (a) is maintained at a pH below 6.

4. A process according to any of the preceding claims, wherein the protein precipitant in step (b) is selected from the group consisting of polyethylene glycol (PEG), caprylic acid, and ammonium sulphate.

5. A process according to claim 4, wherein the protein precipitant is selected from the group consisting of PEG within the molecular weight range 3000-8000 Da, such as PEG 3350, PEG 4000, PEG 5000, and PEG 6000.

6. A process according to any of the preceding claims, wherein the anion exchange resin and the cation exchange resin in step (i) are connected in series.

7. A process according to claim 6, wherein the buffer used for the anion exchange chromatography and the cation exchange chromatography is the same buffer, and the pH of said same buffer is below 6.0.

8. A process according to any of the preceding claims, wherein the anion exchange resin in step (d) and/or step (i) contains diethylaminoethyl groups and/or the cation exchange resin in step (d) and/or step (i) contains carboxymethyl groups, the resins preferably being DEAE Sepharose FF® and CM Sepharose FF®.

9. A process according to any of the preceding claims, wherein the buffer used throughout steps (b) to (l) is an acetate buffer, such as an acetate buffer with a pH of 5.0-6.0 and having a molarity of 5-25 mM.

10. A process according to any of the preceding claims, wherein the virus-inactivating agent in step (h) is a mixture of at least one non-ionic or ionic detergent and at least one solvent.

11. A process according to any of the preceding claims, wherein the virus-inactivating agent in step (h) is a mixture of at least one substantially non-denaturating detergent and at least one tri(lower alkyl) phosphate solvent.

12. An immunoglobulin product which is obtainable by the process according to any of claims 1-11.

13. An immunoglobulin product according to claim 12 having the following characteristics:

a) a purity of more than 98%,
b) a content of IgG monomers and dimers of more than 98.5%,
c) a content of IgA less than 4 mg of IgA/l, and

d) a content of IgG1, IgG2, IgG3 and IgG4.

14. An immunoglobulin product according to claim 13 which does not comprise detergent, PEG or albumin as a stabilizer.

15. An immunoglobulin product according to claim 13 or 14 which contains less than 3 mg/l IgA.

16. An immunoglobulin product according to any of claims 13-15 which contains between 55 and 65% IgG1, between 30 and 40% IgG2, between 2 and 5% IgG3, and between 1 and 4% IgG4.

17. An immunoglobulin product according to any of claims 13-16 which contains less than 0.5% polymers and aggregates.

18. An immunoglobulin product according to any of claims 13-17, which is a liquid.

19. An immunoglobulin product according to any of claims 13-18 for use in medicine.

20. An immunoglobulin product according to claim 19 for instant intravenous administration.

21. Use of an immunoglobulin product according to any of claims 13-20 for the preparation of a medicament for the treatment of a mammal with PID (Primary Immune Deficiency), SID (Secondary Immune Deficiency), ITP (Idiopathic Thrombocytopenic Purpura), polyradiculitis, peripheral polyneuropathies, Kawasaki's disease, polymyositis, severe chronic autoimmune diseases, Chronic inflammatory demyelinating polyneuropathy (CIDP), multifocal motoric neuropathy, multiple sclerosis, Myasthenia Gravis, Eaton-Lambert's syndrome, Opticus Neuritis, epilepsy, Abortus habitualis, primary antiphospholipid syndrome, Rheumatoid arthritis, systemic lupus erythematosus, systemic scleroderma, vasculitis, Wegner's granulomatosis, Sjögren's syndrome, juvenile rheumatoid arthritis, Autoimmune neutropenia, autoimmune haemolytic anaemia, neutropenia, Crohn's disease, colitic ulcerous, coeliac disease, Asthma, septic shock syndrome, chronic fatigue syndrome, psoriasis, toxic shock syndrome, diabetes, sinuitis, dilated cardiomyopathy, endocarditis, atherosclerosis, and adults with AIDS and bacterial infections.

22. Use according to claim 21, wherein the mammal is a human being.

Patentansprüche

1. Verfahren zum Aufreinigen von Immunglobulin G (IgG) aus einer Rohimmunglobulin enthaltenden Plasmaproteinfraktion, wobei das Verfahren die Schritte beinhaltet:

a) Herstellen einer wässrigen Suspension der Rohimmunglobulin enthaltenden Plasmaproteinfraktion;

b) Hinzufügen eines wasserlöslichen, im Wesentlichen nicht-denaturierenden Proteinausfällungsmittel zu der Suspension aus Schritt (a) in einer Menge, die hinreichend ist, um das Ausfällen eines hohen Anteils von nicht-Immunglobulin G-Proteinen, aggregierten Immunglobulinen und Partikeln, einschließlich potenziell infektiösen Partikeln wie Viruspartikeln zu bewirken, ohne eine wesentliche Ausfällung von monomerem Immunglobulin G zu bewirken, wobei eine Mischung aus einem festen Niederschlag und einem flüssigen Überstand gebildet wird;

c) Gewinnen eines gereinigten, Immunglobulin G enthaltenden Überstandes aus der Mischung von Schritt (b);

d) Aussetzen des gereinigten, Immunglobulin G enthaltenden Überstandes aus Schritt (c) einem Anionenaustauscherharz und nachfolgend einem Kationenaustauscherharz, wobei das Anionenaustauscherharz und das Kationenaustauscherharz in Reihe verbunden sind und wobei der Puffer, der für die Anionenaustauschchromatographie und die Kationenaustauschchromatographie verwendet wird, der gleiche Puffer ist, und der pH-Wert des genannten gleichen Puffers geringer als 6,0 ist.

e) Auswaschen von Proteinkontaminanten und dem Proteinfällungsmittel von dem Kationenaustauscherharz von Schritt (d) mit einem Puffer, der einen pH-Wert und eine Ionenstärke besitzt, die hinreichend dafür sind, die Kontaminanten von dem Harz zu entfernen, ohne eine erhebliche Elution von Immunglobulin G zu bewir-

ken;

f) Eluieren von Immunglobulin G von dem Kationenaustauscherharz von Schritt (e) mit einem im Wesentlichen nicht-denaturierenden Puffer, der einen pH-Wert und eine Ionenstärke besitzt, die hinreichend dafür sind, effiziente Elution des Immunglobulin G zu bewirken, wodurch ein Immunglobulin G enthaltendes Eluat gewonnen wird;

g) Durchführen einer Dia-/Ultrafiltration mit dem das Immunglobulin G enthaltenden Eluat aus Schritt (f), um das Eluat zu konzentrieren und/oder zu dialysieren und ggf. Hinzufügen eines stabilisierenden Agens;

h) Hinzufügen einer viruziden Menge eines Virus-inaktivierenden Mittels zu der Immunglobulin G enthaltenden, dia-/ultrafiltrierten und ggf. stabilisierten Fraktion aus Schritt (g), was eine im Wesentlichen Virus-sichere, Immunglobulin G enthaltende Lösung ergibt;

i) Aussetzen der Immunglobulin G enthaltenden Lösung von Schritt (h) einem Anionenaustauscherharz und nachfolgend einem Kationenaustauscherharz;

j) Waschen des Kationenaustauscherharzes aus Schritt (i) mit einem Puffer, der einen pH-Wert und eine Ionenstärke besitzt, die hinreichend dafür sind, die Proteinkontaminationen und das Virusinaktivierende Mittel von dem Harz auszuwaschen, ohne eine erhebliche Elution von Immunglobulin G zu bewirken;

k) Eluieren von Immunglobulin G von dem Kationenaustauscherharz aus Schritt (j) mit einem im Wesentlichen nicht-denaturierenden Puffer, der einen pH-Wert und eine Ionenstärke besitzt, die hinreichend dafür sind, effiziente Elution des Immunglobulin G zu bewirken, wodurch ein Immunglobulin G enthaltendes Eluat gewonnen wird; und

l) Unterwerfen des Immunglobulin G enthaltenden Eluates aus Schritt (k) einer Dia-/Ultraflitration, um die Ionenstärke zu verringem und immunglobulin G aus der Lösung zu konzentrieren, und Einstellen der Osmolalität durch Hinzufügen eines Saccharids.

2. Verfahren nach Anspruch 1, wobei die Immunglobulin G enthaltende Plasmaproteinfraktion ausgewählt ist aus der Gruppe bestehend aus Cohnfraktion II; Cohnfraktionen II und III; und Cohnfraktionen I, II und III.

3. Verfahren nach Anspruch 1 oder 2, wobei die Suspension in Schritt (a) auf einer Temperatur von 0°C bis 12°C gehalten wird und die Suspension in Schritt (a) auf einem pH-Wert unterhalb 6 gehalten wird.

4. Verfahren nach einem der vorhergehenden Ansprüche, wobei das Proteinfällungsmittel in Schritt (b) ausgewählt ist aus der Gruppe bestehend aus Polyethylenglycol (PEG), Caprylsäure und Ammoniumsulfat.

5. Verfahren nach Anspruch 4, wobei das Proteinfällungsmittel ausgewählt ist aus der Gruppe bestehend aus PEG innerhalb des Molekulargewichtbereiches 3000-8000 Da, wie z.B. PEG 3350, PEG 4000, PEG 5000 und PEG 6000.

6. Verfahren nach einem der vorhergehenden Ansprüche, wobei das Anionenaustauscherharz und das Kationenaustauscherharz in Schritt (i) in Reihe verbunden sind.

7. Verfahren nach Anspruch 6, wobei der für die Anionenaustauschchromatographie und die Kationenaustauschchromatographie verwendete Puffer der gleiche Puffer ist und der pH-Wert des genannten gleichen Puffers geringer als 6,0 ist.

8. Verfahren nach einem der vorhergehenden Ansprüche, wobei das Anionenaustauscherharz in Schritt (d) und/oder Schritt (i) Diethylaminoethylgruppen enthält und/oder das Kationenaustauscherharz in Schritt (d) und/oder Schritt (i) Carboxymethylgruppen enthält, wobei die Harze bevorzugt DEAE Sepharose FF® und CM Sepharose FF® sind.

9. Verfahren nach einem der vorhergehenden Ansprüche, wobei der Puffer, der in den Schritten (b) bis (l) verwendet wird, ein Acetatpuffer, wie z.B. ein Acetatpuffer mit einem pH-Wert von 5,0-6,0 und mit einer Molarität von 5-25 mM ist.

10. Verfahren nach einem der vorhergehenden Ansprüche, wobei das Virusinaktivierende Mittel in Schritt (h) eine Mischung von wenigstens einem nicht-ionischen oder ionischen Detergenz und wenigstens einem Lösungsmittel ist

11. Verfahren nach einem der vorhergehenden Ansprüche, wobei das Virusinaktivierende Mittel in Schritt (h) eine Mischung von wenigstens einem im Wesentlichen nicht-denaturierenden Detergenz und wenigstens einem Tri (niederalkyl)phosphatlösungsmittel ist.

12. Immunglobulinerzeugnis, das erhältlich ist mit dem Verfahren nach einem der Ansprüche 1 bis 11.

13. Immunglobulinerzeugnis nach Anspruch 12 mit den folgenden Eigenschaften:

a) eine Reinheit von mehr als 98%,
b) ein Gehalt an IgG-Monomeren und -Dimeren von mehr als 98,5%,
c) ein Gehalt an IgA von weniger als 4 mg IgA/l und
d) ein Gehalt an IgG1, IgG2, IgG3 und IgG4.

14. Immunglobulinerzeugnis nach Anspruch 13, das kein Detergenz, PEG oder Albumin als Stabilisator enthält.

15. Immunglobulinerzeugnis nach Anspruch 13 oder 14, das weniger als 3 mg/l IgA enthält.

16. Immunglobulinerzeugnis nach einem der Ansprüche 13 bis 15, das zwischen 55 und 65% IgG1, zwischen 30 und 40% IgG2, zwischen 2 und 5% IgG3 und zwischen 1 und 4% IgG4 enthält.

17. Immunglobulinerzeugnis nach einem der Ansprüche 13 bis 16, das weniger als 0,5% Polymere und Aggregate enthält.

18. Immunglobulinerzeugnis nach einem der Ansprüche 13 bis 17, das eine Flüssigkeit ist.

19. Immunglobulinerzeugnis nach einem der Ansprüche 13 bis 18 zur Verwendung im Bereich der Medizin.

20. Immunglobulinerzeugnis nach Anspruch 19 zur sofortigen intravenösen Verabreichung.

21. Verwendung eines Immunglobulinerzeugnisses nach einem der Ansprüche 13 bis 20 zur Herstellung eines Medikamentes zur Behandlung eines Säugers mit PID (Primary Immune Deficiency, Primäre Immunschwäche), SID (Secondary Immune Deficiency, Sekundäre Immunschwäche), ITP (i-diopathische thrombocytopenische Purpura), Polyradiculitis, peripheren Polyneuropathien, Kawasaki-Krankheit, Polymyositis, schweren chronischen Autoimmunkrankheiten, chronischer entzündlicher Entmarkungspolyneuropathie (CIDP), multifokaler motorischer Neuropathie, Multipler Sklerose, Myasthenia Gravis, Pseudomyasthenie, Optikusneuritis, Epilepsie, Abortus habitualis, primärem Antiphospholipidsyndrom, rheumatoider Arthritis bzw. Polyarthritis, systemischem Lupus erythematodes, systemischer Sklerodermie, Vaskulitis, Wegener-Granulomatose, Sjögren-Syndrom, juveniler Polyarthritis, Autoimmunneutropenie, autoimmunhaemolytische Anaemie, Neutropenie, Morbus Crohn, Colitis ulcerosa, Zöliakie, Asthma, septischem Schocksyndrom, chronischem Ermüdungssyndrom, Psoriasis, toxischem Schocksyndrom, Diabetes, Sinuitis, dilatierter Kardiomyopathie, Endokarditis, Atherosklerose, und von Erwachsenen mit AIDS und Bakterieninfektionen.

22. Verwendung nach Anspruch 21, wobei der Säuger ein Mensch ist

Revendications

1. Procédé de purification d'immunoglobuline G (IgG), à partir d'une fraction de protéine de plasma brute contenant des immunoglobulines, ledit procédé comprenant les étapes de :

(a) préparation d'une suspension aqueuse d'une fraction de protéine de plasma brute contenant des immunoglobulines ;

(b) addition d'un agent de précipitation de protéine substantiellement non dénaturant, soluble dans l'eau, à

ladite suspension de l'étape (a) en une quantité suffisante pour provoquer une précipitation d'une proportion élevée des protéines non immunoglobulines G, d'immunoglobulines agrégées et de particules comprenant des particules potentiellement infectieuses telles que des particules de virus, sans provoquer de précipitation substantielle de l'immunoglobuline G monomère, formant ainsi un mélange d'un précipité solide et d'un surnageant liquide ;

(c) récupération d'un surnageant clarifié contenant l'immunoglobuline G à partir du mélange de l'étape (b) ;

(d) application du surnageant clarifié contenant l'immunoglobuline G de l'étape (c) sur une résine échangeuse d'anions et ensuite sur une résine échangeuse de cations, pour lesquelles la résine échangeuse d'anions et la résine échangeuse de cations sont reliées en série et pour lesquelles le tampon utilisé pour la chromatographie échangeuse d'anions et pour la chromatographie échangeuse de cations est le même tampon, le pH dudit même tampon étant inférieur à 6,0 ;

(e) élimination par lavage des contaminants protéiniques et de l'agent de précipitation de protéine de la résine échangeuse de cations de l'étape (d) avec un tampon possédant un pH et une force ionique suffisants pour éliminer les contaminants de la résine sans provoquer une élution substantielle de l'immunoglobuline G ;

(f) élution de l'immunoglobuline G à partir de la résine échangeuse de cations de l'étape (e) avec un tampon substantiellement non dénaturant possédant un pH et une force ionique suffisants pour provoquer une élution efficace de l'immunoglobuline G, récupérant ainsi un éluat contenant l'immunoglobuline G ;

(g) réalisation d'une dia/ultrafiltration sur l'éluat contenant l'immunoglobuline G de l'étape (f) pour concentrer et/ou dialyser l'éluat, et éventuellement addition d'un agent de stabilisation ;

(h) addition d'une quantité virucide d'un agent d'inactivation de virus à la fraction dia/ultrafiltrée et éventuellement stabilisée contenant l'immunoglobuline G de l'étape (g) résultant en une solution contenant l'immunoglobuline G substantiellement dépourvue de virus ;

(i) application de la solution contenant l'immunoglobuline G de l'étape (h) sur une résine échangeuse d'anion et ensuite sur une résine échangeuse de cations ;

(j) lavage de la résine échangeuse de cations de l'étape (i) avec un tampon possédant un pH et une force ionique suffisants pour éliminer par lavage les contaminants protéiniques et les agents d'inactivation de virus de la résine sans provoquer une élution substantielle de l'immunoglobuline G ;

(k) élution de l'immunoglobuline G à partir de la résine échangeuse de cations de l'étape (j) avec un tampon substantiellement non dénaturant possédant un pH et une force ionique suffisants pour provoquer une élution efficace de l'immunoglobuline G, récupérant ainsi un éluat contenant l'immunoglobuline G ; et

(l) application à l'éluat contenant l'immunoglobuline G de l'étape (k) d'une dia/ultrafiltration pour diminuer la force ionique et concentrer l'immunoglobuline G de la solution, et ajustement de l'osmolalité par addition d'un saccharide.

2. Procédé selon la revendication 1, dans lequel la fraction de protéine de plasma contenant l'immunoglobuline G est choisie dans le groupe consistant en une fraction II de Cohn ; des fractions II et III de Cohn ; et des fractions I, II et III de Cohn.

3. Procédé selon la revendication 1 ou 2, dans lequel la suspension de l'étape (a) est maintenue à une température comprise entre 0°C et 12°C et la suspension de l'étape (a) est maintenue à un pH inférieur à 6.

4. Procédé selon l'une quelconque des revendications précédentes, dans lequel l'agent de précipitation de protéine de l'étape (b) est choisi dans le groupe consistant en du polyéthylène glycol (PEG), de l'acide caprylique et du sulfate d'ammonium.

5. Procédé selon la revendication 4, dans lequel l'agent de précipitation de protéine est choisi dans le groupe consistant en un PEG de poids moléculaire compris dans la gamme de 3000 à 8000 Da, tel que PEG 3350, PEG 4000, PEG 5000, et PEG 6000.

6. Procédé selon l'une quelconque des revendications précédentes, dans lequel la résine échangeuse d'anions et la résine échangeuse de cations de l'étape (i) sont connectées en séries.

7. Procédé selon la revendication 6, dans lequel le tampon utilisé pour la chromatographie échangeuse d'anions et pour la chromatographie échangeuse de cations est le même tampon, et le pH dudit même tampon est inférieur à 6,0.

8. Procédé selon l'une quelconque des revendications précédentes, dans lequel la résine échangeuse d'anions de l'étape (d) et/ou de l'étape (i) contient des groupes diéthylaminoéthyle et/ou la résine échangeuse de cations de l'étape (d) et/ou de l'étape (i) contient des groupes carboxyméthyle, les résines étant de préférence DEAE Sepharose FF® et CM Sepharose FF®.

9. Procédé selon l'une quelconque des revendications précédentes, dans lequel le tampon utilisé au cours des étapes (b) à (1) est un tampon acétate, tel qu'un tampon acétate possédant un pH de 5,0 à 6,0 et possédant une molarité de 5 à 25 mM.

10. Procédé selon l'une quelconque des revendications précédentes, dans lequel l'agent d'inactivation de virus de l'étape (h) est un mélange d'au moins un détergent non ionique ou ionique et au moins un solvant.

11. Procédé selon l'une quelconque des revendications précédentes, dans lequel l'agent d'inactivation de virus de l'étape (h) est un mélange d'au moins un détergent substantiellement non dénaturant et au moins un solvant tri (alkyl inférieur) phosphate.

12. Produit d'immunoglobulines susceptible d'être obtenu par le procédé selon l'une quelconque des revendications 1 à 11.

13. Produit d'immunoglobulines selon la revendication 12 possédant les caractéristiques suivantes :

a) une pureté supérieure à 98%,
b) une teneur en monomères et dimères d'IgG supérieure à 98,5%,
c) une teneur en IgA inférieure à 4 mg d'IgA/l, et
d) une teneur en IgG1, IgG2, IgG3 et IgG4.

14. Produit d'immunoglobulines selon la revendication 13 qui ne comprend pas de détergent, de PEG ou d'albumine comme stabilisant.

15. Produit d'immunoglobulines selon la revendication 13 ou 14 qui contient moins de 3 mg/l d'IgA.

16. Produit d'immunoglobulines selon l'une quelconque des revendications 13 à 15 qui contient entre 55 et 65% d'IgG1, entre 30 et 40% d'IgG2, entre 2 et 5% d'IgG3, et entre 1 et 4% d'IgG4.

17. Produit d'immunoglobulines selon l'une quelconque des revendications 13 à 16 qui contient moins de 0,5% de polymères et d'agrégats.

18. Produit d'immunoglobulines selon l'une quelconque des revendications 13 à 17 qui est un liquide.

19. Produit d'immunoglobulines selon l'une quelconque des revendications 13 à 18 pour une utilisation en médecine.

20. Produit d'immunoglobulines selon la revendication 19 pour administration intraveineuse immédiate.

21. Utilisation d'un produit d'immunoglobulines selon l'une quelconque des revendications 13 à 20 pour la préparation d'un médicament pour le traitement d'un mammifère présentant une PID (Immunodéficience Primaire), une SID (Immunodéficience secondaire), un PTI (Purpura Thrombocytopénique Idiopathique), une polyradiculite, des polynévrites, une maladie de Kawasaki, une polymyosite, des maladies auto-immunes chroniques sévères, une névropathie démyélinisante inflammatoire chronique (CIDP), une névropathie motrice multifocale, une sclérose en plaques, une myasthénie Gravis, un syndrome de Eaton-Lambert, une névrite optique, une épilepsie, un Abortus habitualis, un syndrome antiphospholipide primaire, une arthrite rhumatoïde, un lupus érythémateux systémique, une sclérodermie systémique, une vascularite, une granulomatose de Wegner, un syndrome de Sjögren, une

arthrite rhumatoïde juvénile, une neutropénie auto-immune, une anémie hémolytique autoimmune, une neutropénie, une maladie de Crohn, une colite ulcéreuse, une maladie coeliaque, un asthme, un syndrome de choc septique, un syndrome de fatigue chronique, un psoriasis, un syndrome de choc toxique, un diabète, une sinusite, une cardiomyopathie dilatée, une endocardite, une athérosclérose, et des infections bactériennes et de SIDA chez l'adulte.

22. Utilisation selon la revendication 21, dans laquelle le mammifère est un être humain.

HIGHLIGHTS OF PRESCRIBING INFORMATION

These highlights do not include all the information needed to use Privigen™ safely and effectively. See full prescribing information for Privigen™.

Immune Globulin Intravenous (Human), 10% Liquid
Privigen™
Initial U.S. Approval: XXXX

WARNING: ACUTE RENAL DYSFUNCTION/FAILURE
See full prescribing information for complete boxed warning.

- **Renal dysfunction, acute renal failure, osmotic nephrosis, and death may be associated with the administration of Immune Globulin Intravenous (Human) (IGIV) products in predisposed patients.**
- **Administer IGIV products at the minimum infusion rate practicable.**
- **Renal dysfunction and acute renal failure occur more commonly in patients receiving IGIV products containing sucrose. Privigen™ does not contain sucrose.**

----------INDICATIONS AND USAGE----------

Privigen™ is an Immune Globulin Intravenous (Human), 10% Liquid indicated for treatment of:

- Primary immunodeficiency (PI) (1.1)
- Chronic immune thrombocytopenic purpura (ITP) (1.2)

----------DOSAGE AND ADMINISTRATION----------

- **PI** – 200 to 800 mg/kg intravenously (IV) every 3 to 4 weeks. Recommended infusion rate: initially, 0.5 mg/kg/min (0.005 mL/kg/min); if well tolerated, may be gradually increased to 8 mg/kg/min (0.08 mL/kg/min) (2.2).
- **Chronic ITP** – 1 g/kg IV daily for 2 consecutive days, for a total of 2 g/kg. Recommended infusion rate: initially, 0.5 mg/kg/min (0.005 mL/kg/min); if well tolerated, may be gradually increased to 4 mg/kg/min (0.04 mL/kg/min) (2.3).
- Ensure that patients with pre-existing renal insufficiency are not volume depleted; discontinue Privigen™ if renal function deteriorates (5.1).
- For patients at risk of renal dysfunction or thrombotic events, administer Privigen™ at the minimum infusion rate practicable (5.1, 5.5).

----------DOSAGE FORMS AND STRENGTHS----------

5 g in 50 mL solution, 10 g in 100 mL solution, 20 g in 200 mL solution (3)

----------CONTRAINDICATIONS----------

- Anaphylactic or severe systemic reactions to human immunoglobulin (4)
- Hyperprolinemia (Privigen™ contains the stabilizer L-proline) (4)
- Individuals with selective IgA deficiency can develop antibodies to IgA and are at greater risk of developing severe hypersensitivity and anaphylactic reactions (4)

----------WARNINGS AND PRECAUTIONS----------

- Monitor renal function, including blood urea nitrogen and serum creatinine, and urine output in patients at risk of developing acute renal failure (5.1).
- Aseptic meningitis syndrome has been reported with Privigen™ and other IGIV treatments, especially with high doses or rapid infusion (5.2).
- Hemolysis has been reported with Privigen™ and other IGIV treatments. Monitor patients for hemolysis and hemolytic anemia (5.3).
- Monitor patients for pulmonary adverse reactions; if transfusion-related acute lung injury is suspected, test the product and patient for antineutrophil antibodies (5.4).
- Thrombotic events have been reported with Privigen™ and other IGIV treatments. Monitor patients with known risk factors for thrombotic events; consider baseline assessment of blood viscosity for those at risk of hyperviscosity (5.5).
- Products made from human plasma can contain infectious agents, e.g., viruses and, theoretically, the Creutzfeldt-Jakob disease agent (5.6).

----------ADVERSE REACTIONS----------

- **PI** – Most common adverse reactions are headache, pain, nausea, fatigue, and chills (6.1).
- **Chronic ITP** – Most common adverse reactions are headache, pyrexia/hyperthermia, and anemia (6.1).

To report SUSPECTED ADVERSE REACTIONS, contact CSL Behring at 1-800-504-5434 or FDA at 1-800-FDA-1088 or *www.fda.gov/medwatch.*

----------DRUG INTERACTIONS----------

IgG administration can transiently impair efficacy of live virus vaccines (7.1).

----------USE IN SPECIFIC POPULATIONS----------

In patients over age 65 at risk of developing renal insufficiency, do not exceed the recommended dose, and infuse Privigen™ at a rate less than 2 mg/kg/min (0.02 mL/kg/min) (8.5).

See 17 for PATIENT COUNSELING INFORMATION.

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* Sections or subsections omitted from the full prescribing information are not listed.

FULL PRESCRIBING INFORMATION

Immune Globulin Intravenous (Human), 10% Liquid Privigen™

WARNING: ACUTE RENAL DYSFUNCTION AND ACUTE RENAL FAILURE

Immune Globulin Intravenous (Human) (IGIV) products have been reported to be associated with renal dysfunction, acute renal failure, osmotic nephrosis, and death.[1] Patients predisposed to acute renal failure include patients with any degree of pre-existing renal insufficiency, diabetes mellitus, age greater than 65, volume depletion, sepsis, paraproteinemia, or patients receiving known nephrotoxic drugs. In such patients, IGIV products should be administered at the minimum rate of infusion practicable. While these reports of renal dysfunction and acute renal failure have been associated with the use of many of the licensed IGIV products, those containing sucrose as a stabilizer accounted for a disproportionate share of the total number. Privigen™ does not contain sucrose. (*See Dosage and Administration [2.4] and Warnings and Precautions [5.1] for important information intended to reduce the risk of acute renal failure.*)

1 INDICATIONS AND USAGE

1.1 Treatment of Primary Immunodeficiency

Privigen™ is indicated for the treatment of patients with primary immunodeficiency (PI) associated with defects in humoral immunity. This includes, but is not limited to, common variable immunodeficiency (CVID), X-linked agammaglobulinemia, congenital agammaglobulinemia, Wiskott-Aldrich syndrome, and severe combined immunodeficiencies.

1.2 Treatment of Chronic Immune Thrombocytopenic Purpura

Privigen™ is indicated for the treatment of patients with chronic immune thrombocytopenic purpura (ITP) to rapidly raise platelet counts to prevent bleeding.

2 DOSAGE AND ADMINISTRATION

2.1 Preparation and Handling

Privigen™ is a clear or slightly opalescent, colorless to pale yellow solution. Privigen™ should be inspected visually for particulate matter and discoloration prior to administration. Do not use if the solution is cloudy or contains particulates. Any solution that has been frozen must not be used. DO NOT SHAKE.

Do not mix Privigen™ with other IGIV products or other intravenous medications. If necessary, Privigen™ can be diluted with Dextrose Injection, USP (D5W). If large doses of Privigen™ are to be administered, several vials may be pooled using aseptic technique.

The Privigen™ vial is for single use only. Once the vial has been entered under aseptic conditions, its contents should be used promptly. Because the solution contains no preservative, Privigen™ should be infused as soon as possible. Any unused product or waste material should be disposed of in accordance with local requirements.

2.2 Treatment of Primary Immunodeficiency

The usual dose of Privigen™ for patients with PI is 200 to 800 mg/kg, administered every 3 to 4 weeks. An optimum target serum immunoglobulin G (IgG) trough level has not been established in randomized, controlled clinical studies. Doses should be adjusted to achieve the desired serum trough levels and clinical responses.

The recommended initial infusion rate is 0.5 mg/kg/min (0.005 mL/kg/min). If the infusion is well tolerated, the rate may be gradually increased to a maximum of 8 mg/kg/min (0.08 mL/kg/min). For patients judged to be at risk of renal dysfunction or thrombotic events, Privigen™ should be administered at the minimum infusion rate practicable (*see Warnings and Precautions [5.1, 5.5]*).

2.3 Treatment of Chronic Immune Thrombocytopenic Purpura

The usual dose of Privigen™ for patients with chronic ITP is 1 g/kg administered daily for 2 consecutive days, resulting in a total dosage of 2 g/kg.

The recommended initial infusion rate is 0.5 mg/kg/min (0.005 mL/kg/min). If the infusion is well tolerated, the rate may be gradually increased to a maximum of 4 mg/kg/min (0.04 mL/kg/min). For patients judged to be at risk of renal dysfunction or thrombotic events, Privigen™ should be administered at the minimum infusion rate practicable (*see Warnings and Precautions [5.1, 5.5]*).

2.4 Administration

Privigen™ is for intravenous (IV) administration and should be given by a separate infusion line. An infusion pump may be used to control the rate of administration. The Privigen™ infusion line can be flushed with Dextrose Injection, USP (D5W) or 0.9% Sodium Chloride for Injection, USP.

The following patients may be at risk of developing inflammatory reactions on rapid infusion of Privigen™ (greater than 4 mg/kg/min [0.04 mL/kg/min]): 1) those who have not received Privigen™ or another IgG product; 2) those who are switching from another IgG product; and 3) those who have not received IgG in more than 8 weeks. These patients should be started at a slow rate of infusion (e.g., 0.5 mg/kg/min [0.005 mL/kg/min] or less) and gradually advanced to the maximum rate as tolerated.

Ensure that patients with pre-existing renal insufficiency and those predisposed to acute renal failure are not volume depleted before administering Privigen™ (*see Boxed Warning, Warnings and Precautions [5.1]*).

The patient's vital signs should be observed and monitored carefully throughout the infusion. If side effects occur, the infusion should be slowed or stopped until the symptoms subside. The infusion may then be resumed at a lower rate that is comfortable for the patient.

3 DOSAGE FORMS AND STRENGTHS

5 g in 50 mL solution
10 g in 100 mL solution
20 g in 200 mL solution

4 CONTRAINDICATIONS

Privigen™ is contraindicated in patients who have had an anaphylactic or severe systemic reaction to the administration of human immune globulin.

Because it contains the stabilizer L-proline, Privigen™ is contraindicated in patients with hyperprolinemia.

Privigen™ is contraindicated in individuals with selective IgA deficiency because they can develop antibodies to IgA and anaphylactic reactions (including anaphylaxis and shock) after administration of blood components containing IgA. Privigen™ contains trace amounts of IgA (*see Description [11]*).

5 WARNINGS AND PRECAUTIONS

5.1 Acute Renal Dysfunction and Acute Renal Failure

Patients should not be volume depleted prior to the initiation of the infusion of Privigen™. Periodic monitoring of renal function and urine output is particularly important in patients judged to have a potential increased risk of developing acute renal failure. Renal function, including measurement of blood urea nitrogen (BUN) and serum creatinine, should be assessed before the initial infusion of Privigen™ and at appropriate intervals thereafter. For patients judged to be at risk of developing renal dysfunction, Privigen™ should be administered at the minimum rate of infusion practicable (*see Dosage and Administration [2.2, 2.3]*). If renal function deteriorates, consider discontinuing Privigen™. (*See Patient Counseling Information [17.1]*.)

5.2 Aseptic Meningitis Syndrome (AMS)

AMS has been reported to occur infrequently with Privigen™ and other IGIV treatments. The syndrome usually begins within several hours to 2 days following IGIV treatment. AMS is characterized by signs and symptoms including severe headache, nuchal rigidity, drowsiness, fever, photophobia, painful eye movements, nausea, and vomiting. Cerebrospinal fluid (CSF) studies are frequently positive with pleocytosis up to several thousand cells per cubic millimeter, predominantly from the granulocytic series, and with elevated protein levels up to several hundred mg/dL. Patients exhibiting such signs and symptoms should receive a thorough neurological examination, including CSF studies, to rule out other causes of meningitis. AMS may occur more frequently in association with high doses (2 g/kg) and/or rapid infusion of IGIV. Discontinuation of IGIV treatment has resulted in remission of AMS within several days without sequelae.[2] (*See Patient Counseling Information [17.2].*)

5.3 Hemolysis

IGIV products can contain blood group antibodies that may act as hemolysins and induce *in vivo* coating of red blood cells (RBCs) with immunoglobulin, causing a positive direct antiglobulin reaction and, rarely, hemolysis.[3-5] Hemolytic anemia can develop subsequent to IGIV therapy due to enhanced RBC sequestration (extravascular hemolysis) or intravascular RBC destruction (intravascular hemolysis).[6]

Hemolysis, possibly intravascular, occurred in two subjects treated with Privigen™ in the ITP study. These cases resolved uneventfully. Six other subjects experienced hemolysis in the ITP study as documented from clinical laboratory data.

IGIV recipients should be monitored for clinical signs and symptoms of hemolysis (*see Patient Counseling Information [17.3]*). If signs and/or symptoms of hemolysis are present after IGIV infusion, appropriate confirmatory laboratory testing should be performed. If transfusion is indicated for patients who develop hemolysis with clinically compromising anemia after receiving IGIV, adequate cross-matching should be performed to avoid exacerbating on-going hemolysis.

5.4 Transfusion-related Acute Lung Injury (TRALI)

There have been reports of noncardiogenic pulmonary edema in patients administered IGIV.[7] TRALI is characterized by severe respiratory distress, pulmonary edema, hypoxemia, normal left ventricular function, and fever and typically occurs within 1 to 6 hours following transfusion. IGIV recipients should be monitored for pulmonary adverse reactions (*see Patient Counseling Information [17.4]*). Patients with TRALI may be managed using oxygen therapy with adequate ventilatory support.

If TRALI is suspected, appropriate tests should be performed for the presence of antineutrophil antibodies in both the product and the patient's serum.

5.5 Thrombotic Events

Thrombotic events have been reported with Privigen™ and other IGIV treatments.[8-10] Patients at risk may include those with a history of atherosclerosis, multiple cardiovascular risk

factors, advanced age, impaired cardiac output, hypercoagulable disorders, prolonged periods of immobilization, and/or known or suspected hyperviscosity. The potential risks and benefits of IGIV should be weighed against those of alternative therapies in all patients for whom IGIV administration is being considered.

Because of the potentially increased risk of thrombosis, baseline assessment of blood viscosity should be considered in patients at risk of hyperviscosity, including those with cryoglobulins, fasting chylomicronemia/markedly high triacylglycerols (triglycerides), or monoclonal gammopathies.

5.6 Transmissible Infectious Agents

Privigen™ is made from human plasma. Products made from human plasma may contain infectious agents, e.g., viruses, and theoretically the Creutzfeldt-Jakob disease (CJD) agent, that can cause disease. The risk that such products will transmit an infectious agent has been reduced by screening plasma donors for prior exposure to certain viruses, by testing for the presence of certain current virus infections, and by inactivating and/or removing certain viruses during manufacturing through pH 4 incubation, depth filtration, and virus filtration (*see Description [11]*).

Despite these measures, such products can still potentially transmit disease. There is also the possibility that unknown infectious agents may be present in such products. All infections thought by a physician possibly to have been transmitted by this product should be reported by the physician or other healthcare provider to CSL Behring at 1-800-504-5434. (*See Patient Counseling Information [17.5].*)

5.7 Interference With Laboratory Tests

After infusion of IgG, the transitory rise of the various passively transferred antibodies in the patient's blood may yield positive serological testing results, with the potential for misleading interpretation. Passive transmission of antibodies to erythrocyte antigens (e.g., A, B, and D) may cause a positive direct or indirect antiglobulin (Coombs') test.

5.8 Interference With Live Virus Vaccines

Immunoglobulin administration may transiently impair the efficacy of live virus vaccines such as measles, mumps, and rubella. The immunizing physician should be informed so that appropriate measures may be taken (*see Drug Interactions [7.1], Patient Counseling Information [17.6]*).

6 ADVERSE REACTIONS

The most serious adverse reaction observed in clinical study subjects receiving Privigen™ for PI was hypersensitivity in one subject. The most serious adverse reactions observed in subjects receiving Privigen™ for chronic ITP were aseptic meningitis syndrome in one subject and hemolysis in two subjects. Six other subjects in the ITP study experienced hemolysis as documented from clinical laboratory data. (*See Warnings and Precautions [5.2, 5.3]*).

The most common adverse reactions observed in subjects with PI were headache, pain, nausea, fatigue, and chills. The most common adverse reactions observed in subjects with chronic ITP were headache, pyrexia/hyperthermia, and anemia.

In general, reported adverse reactions to Privigen™ in subjects with either PI or chronic ITP were similar in kind and frequency to those observed with other IGIV products.

6.1 Clinical Studies Experience

Because clinical studies are conducted under widely varying conditions, adverse reaction rates observed cannot be directly compared to rates in other clinical trials and may not reflect the rates observed in practice.

Treatment of Primary Immunodeficiency

In a prospective, open-label, single-arm, multicenter clinical study, 80 subjects with PI received median doses of Privigen™ ranging from 200 to 888 mg/kg every 3 weeks (median dose 428.3 mg/kg) or 4 weeks (median dose 440.6 mg/kg) for up to 12 months (*see Clinical Studies [14.1]*).

Routine premedication was not allowed. However, subjects who experienced two consecutive infusion-related adverse events (AEs) that were likely to be prevented by premedication were permitted to receive antipyretics, antihistamines, NSAIDs, or antiemetic agents. During the study, 8 (10%) subjects received premedication prior to 51 (4.9%) of the 1038 infusions administered.

Temporally associated AEs are those occurring during or within 72 hours after the end of an infusion, *irrespective of causality*. In this study, the upper bound of the 1-sided 97.5% confidence interval for the proportion of Privigen™ infusions with temporally associated AEs was 23.8% (actual proportion: 20.8%). This is below the target of 40% for this safety endpoint.[11] The total number of temporally associated AEs was 397 (a rate of 0.38 AEs per infusion).

Table 1 lists the temporally associated AEs that occurred in more than 5% of subjects within 72 hours after the end of a Privigen™ infusion, *irrespective of causality*.

Table 1: Temporally Associated Adverse Events* (TAAEs) in >5% of Subjects With PI Within 72 Hours After the End of a Privigen™ Infusion, *Irrespective of Causality*

TAAE	No. Subjects Reporting TAAE (% of Subjects [n=80])	No. TAAEs Reported (as % Rate of Infusions [n=1038])	No. Infusions With TAAE (% of Infusions [n=1038])
Headache	35 (43.8)	90 (8.7)	82 (7.9)
Pain	20 (25.0)	51 (4.9)	44 (4.2)
Fatigue	13 (16.3)	29 (2.8)	27 (2.6)
Nausea	10 (12.5)	22 (2.1)	19 (1.8)
Chills	9 (11.3)	15 (1.4)	15 (1.4)
Vomiting	7 (8.8)	13 (1.3)	13 (1.3)
Pyrexia	6 (7.5)	11 (1.1)	10 (1.0)
Cough	5 (6.3)	5 (0.5)	5 (0.5)
Diarrhea	5 (6.3)	5 (0.5)	5 (0.5)
Stomach discomfort	5 (6.3)	5 (0.5)	5 (0.5)

*Excluding infections.

Of the 397 temporally associated AEs reported for the 80 subjects with PI, the investigators judged 192 to be related to the infusion of Privigen™ (including 5 serious, severe AEs described below). Of the 187 non-serious AEs related to the infusion of Privigen™, 91 were mild, 81 were moderate, 14 were severe, and 1 was of unknown severity. The most common temporally associated AEs judged by the investigators to be "at least possibly" related to the infusion were headache (29% of subjects), pain (14% of subjects), nausea (11% of subjects), fatigue (11% of subjects), and chills (11% of subjects).

Sixteen subjects (20%) experienced 41 serious AEs. Five of these were related severe AEs (hypersensitivity, chills, fatigue, dizziness, and increased body temperature) that occurred in one subject and resulted in the subject's withdrawal from the study. Two other subjects withdrew from the study due to AEs related to Privigen™ (chills and headache in one subject; vomiting in the other).

Seventy-seven of the 80 subjects enrolled in this study had a negative direct antiglobulin test (DAT) at baseline. Of these 77 subjects, 36 (46.8%) developed a positive DAT at some time during the study. However, no subjects showed evidence of hemolytic anemia.

During this study, no subjects tested positive for infection due to human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV), or B19 virus (B19V).

Treatment of Chronic Immune Thrombocytopenic Purpura

In a prospective, open-label, single-arm, multicenter clinical study, 57 subjects with chronic ITP received a 2 g/kg dose of Privigen™ administered daily as two 1 g/kg intravenous infusions for 2 consecutive days (*see Clinical Studies [14.2]*).

Concomitant medications affecting platelets or other treatments for chronic ITP were not allowed. Thirty-two (56.1%) subjects received premedication with acetaminophen and/or an antihistamine.

Table 2 lists the temporally associated AEs that occurred in more than 5% of subjects with chronic ITP within 72 hours after the end of a treatment cycle (two consecutive infusions) with Privigen™, *irrespective of causality*.

Table 2: Temporally Associated Adverse Events (TAAEs) in >5% Subjects With Chronic ITP Within 72 hours After the End of a Treatment Cycle* With Privigen™, *Irrespective of Causality*

TAAE	No. Subjects Reporting TAAE (% of Subjects [n=57])	No. TAAEs Reported (as % Rate of Infusions [n=114])	No. Infusions With TAAE (% of Infusions [n=114])
Headache	37 (64.9)	48 (42.1)	41 (36.0)
Pyrexia/hyperthermia	21 (36.8)	23 (20.2)	22 (19.3)
Nausea	6 (10.5)	8 (7.0)	6 (5.3)
Epistaxis	6 (10.5)	8 (7.0)	6 (5.3)
Vomiting	6 (10.5)	7. (6.1)	6 (5.3)
Blood unconjugated bilirubin increased	6 (10.5)	6 (5.3)	6 (5.3)
Blood conjugated bilirubin increased	5 (8.8)	5 (4.4)	5 (4.4)
Blood total bilirubin increased	4 (7.0)	4 (3.5)	4 (3.5)
Hematocrit decreased	3 (5.3)	3 (2.6)	3 (2.6)

* Two consecutive daily infusions.

Of the 183 temporally associated AEs reported for the 57 subjects with chronic ITP, the investigators judged 150 to be related to the infusion of Privigen™ (including the one serious AE described below). Of the 149 non-serious AEs related to the infusion of Privigen™, 103 were mild, 37 were moderate, and 9 were severe. The most common temporally associated AEs judged by the investigators to be "at least possibly" related to the infusion were headache (65% of subjects) and pyrexia/hyperthermia (35% of subjects).

Three subjects experienced three serious AEs, one of which (aseptic meningitis) was related to the infusion of Privigen™.

One subject withdrew from the study due to gingival bleeding, which was not related to Privigen™.

Eight subjects, all of whom had a positive DAT, experienced transient drug-related hemolytic reactions, which were associated with elevated bilirubin, elevated lactate dehydrogenase, and a decrease in hemoglobin level within two days after the infusion of Privigen™. Two of the eight subjects were clinically anemic but did not require clinical intervention.

Four other subjects with active bleeding were reported to have developed anemia without evidence of hemolysis.

In this study, there was a decrease in hemoglobin after the first Privigen™ infusion (median decrease of 1.2 g/dL by Day 8) followed by a return to near baseline by Day 29.

Fifty-six of the 57 subjects in this study had a negative DAT at baseline. Of these 56 subjects, 12 (21.4%) developed a positive DAT during the 29-day study period.

6.2 Postmarketing Experience

The following mild to moderate reactions may occur with the administration of IGIV products: headache, diarrhea, tachycardia, fever, fatigue, dizziness, malaise, chills, flushing, skin reactions, wheezing or chest tightness, nausea, vomiting, rigors, back pain, chest pain, myalgia, arthralgia, and changes in blood pressure. Immediate hypersensitivity and anaphylactic reactions are also a possibility.

The following adverse reactions have been identified and reported during the postapproval use of IGIV products.[12]

- *Respiratory*: Apnea, Acute Respiratory Distress Syndrome (ARDS), TRALI, cyanosis, hypoxemia, pulmonary edema, dyspnea, bronchospasm
- *Cardiovascular:* Cardiac arrest, thromboembolism, vascular collapse, hypotension
- *Neurological:* Coma, loss of consciousness, seizures, tremor
- *Integumentary:* Stevens-Johnson syndrome, epidermolysis, erythema multiforme, bullous dermatitis
- *Hematologic:* Pancytopenia, leukopenia, hemolysis, positive direct antiglobulin (Coombs') test
- *General/Body as a Whole:* Pyrexia, rigors
- *Musculoskeletal:* Back pain
- *Gastrointestinal:* Hepatic dysfunction, abdominal pain

Because postmarketing reporting of adverse reactions is voluntary and from a population of uncertain size, it is not always possible to reliably estimate the frequency of these reactions or establish a causal relationship to product exposure. Evaluation and interpretation of these postmarketing reactions is confounded by underlying diagnosis, concomitant medications, pre-existing conditions, and inherent limitations of passive surveillance.

7 DRUG INTERACTIONS

7.1 Live Virus Vaccines

Immunoglobulin administration may transiently impair the efficacy of live attenuated virus vaccines such as measles, mumps, and rubella because the continued presence of high levels of passively acquired antibody may interfere with an active antibody response.[13] The immunizing physician should be informed of recent therapy with Privigen™ so that appropriate measures may be taken (*see Patient Counseling Information [17.6]*).

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Pregnancy Category C. Animal reproduction studies have not been conducted with Privigen™. It is not known whether Privigen™ can cause fetal harm when administered to a pregnant woman or can affect reproduction capacity. Privigen™ should be given to pregnant women only if clearly needed. Immunoglobulins cross the placenta from maternal circulation increasingly after 30 weeks of gestation.[14,15]

8.3 Nursing Mothers

Privigen™ has not been evaluated in nursing mothers.

8.4 Pediatric Use

Treatment of Primary Immunodeficiency

Privigen™ was evaluated in 19 children and 12 adolescents with PI. There were no apparent differences in the safety and efficacy profiles as compared to adult subjects. No pediatric-specific dose requirements were necessary to achieve the desired serum IgG levels. The safety and effectiveness of Privigen™ has not been established in pediatric subjects with PI who are under the age of 3.

Treatment of Chronic Immune Thrombocytopenic Purpura

The safety and effectiveness of Privigen™ has not been established in pediatric subjects with chronic ITP who are under the age of 15.

8.5 Geriatric Use

Privigen™ should be used with caution in patients over 65 years of age who are judged to be at increased risk of developing renal insufficiency (*see Boxed Warning, Warnings and*

Precautions [5.1]). Recommended doses should not be exceeded, and the infusion rate selected should be the minimum practicable. Privigen™ should be infused at a rate less than 2 mg/kg/min (0.02 mL/kg/min).

Clinical studies of Privigen™ did not include sufficient numbers of subjects age 65 and over to determine whether they respond differently from younger subjects.

11 DESCRIPTION

Privigen™ is a ready-to-use, sterile, 10% protein liquid preparation of polyvalent human immunoglobulin G (IgG) for intravenous administration. Privigen™ is prepared from large pools of human plasma by a combination of cold ethanol fractionation, octanoic acid fractionation, and anion exchange chromatography. The IgG proteins are not subjected to heating or to chemical or enzymatic modification. The Fc and Fab functions of the IgG molecule are retained. Fab functions tested include antigen binding capacities, and Fc functions tested include complement activation and Fc-receptor-mediated leukocyte activation (determined with complexed IgG). Privigen™ does not activate the complement system or prekallikrein in an unspecific manner.

Privigen™ has a purity of at least 98% IgG, consisting primarily of monomers. The balance consists of IgG dimers (≤12%), small amounts of fragments and polymers, and albumin. Privigen™ contains ≤25 mcg/mL IgA. The IgG subclass distribution (approximate mean values) is IgG_1, 67.8%; IgG_2, 28.7%; IgG_3, 2.3%; and IgG_4, 1.2%. Privigen™ has an osmolality of approximately 320 mOsmol/kg (range: 240 to 440) and a pH of 4.8 (range: 4.6 to 5.0).

Privigen™ contains approximately 250 mmol/L (range: 210 to 290) of L-proline (a nonessential amino acid) as a stabilizer and trace amounts of sodium. Privigen™ contains no carbohydrate stabilizers (e.g., sucrose, maltose) and no preservative.

All plasma units used in the manufacture of Privigen™ are tested using FDA-licensed serological assays for hepatitis B surface antigen and antibodies to HCV and HIV-1/2 as well as FDA-licensed Nucleic Acid Testing (NAT) for HCV and HIV-1 and found to be nonreactive (negative). For HBV, an investigational NAT procedure is used and the plasma units found to be negative; however, the significance of a negative result has not been established.

The manufacturing process for Privigen™ includes three steps to reduce the risk of virus transmission. Two of these are dedicated virus clearance steps: pH 4 incubation to inactivate enveloped viruses and virus filtration to remove, by size exclusion, both enveloped and non-enveloped viruses as small as approximately 20 nanometers. In addition, a depth filtration step contributes to the virus reduction capacity.

These steps have been independently validated in a series of *in vitro* experiments for their capacity to inactivate and/or remove both enveloped and non-enveloped viruses. Table 3 shows the virus clearance during the manufacturing process for Privigen™, expressed as the mean $\log_{10}$ reduction factor (LRF).

Table 3: Virus Inactivation/Removal in Privigen™

	HIV-1	PRV	BVDV	WNV	EMCV	MVM
Virus property						
Genome	RNA	DNA	RNA	RNA	RNA	DNA
Envelope	Yes	Yes	Yes	Yes	No	No
Size (nm)	80-100	120-200	50-70	50-70	25-30	18-24
Manufacturing step	**Mean LRF**					
pH 4 incubation	≥5.4	≥5.9	4.6	≥7.8	nt	nt
Depth filtration	≥5.3	≥6.3	2.1	3.0	4.2	2.3
Virus filtration	≥5.3	nd	≥2.7	≥5.9	≥3.7	≥5.5
Overall reduction (log_{10} units)	**≥16.0**	**≥12.2**	**≥9.4**	**≥16.7**	**≥7.9**	**≥7.8**

HIV-1, human immunodeficiency virus type 1, a model for HIV-1 and HIV-2; PRV, pseudorabies virus, a nonspecific model for large enveloped DNA viruses (e.g., herpes virus); BVDV, bovine viral diarrhea virus, a model for hepatitis C virus; WNV, West Nile virus; EMCV, encephalomyocarditis virus, a model for hepatitis A virus; MVM, minute virus of mice, a model for a small highly resistant non-enveloped DNA virus (e.g., parvovirus); LRF, log_{10} reduction factor; nd, not determined; nt, not tested.

The manufacturing process was also investigated for its capacity to decrease the infectivity of an experimental agent of TSE, considered a model for CJD and its variant vCJD.[16] Several of the production steps have been shown to decrease TSE infectivity of an experimental model agent. TSE reduction steps include octanoic acid fractionation (≥6.4 log_{10}), depth filtration (2.6 log_{10}), and virus filtration (≥5.8 log_{10}). These studies provide reasonable assurance that low levels of vCJD/CJD agent infectivity, if present in the starting material, would be removed.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Treatment of Primary Immunodeficiency

Privigen™ contains a broad spectrum of antibody specificities. Appropriate doses of Privigen™ should restore abnormally low IgG levels to the normal range.

Treatment of Chronic Immune Thrombocytopenic Purpura

The mechanism of action of immunoglobulins in the treatment of chronic ITP is not fully understood. One possible mechanism may be the inhibition of the elimination of autoantibody-reacted platelets from the blood circulation by IgG-induced Fc-receptor blockade of phagocytes.[17] Another proposed mechanism is the down-regulation of platelet autoantibody-producing B cells by anti-idiotypic antibodies in IGIV.[18]

12.3 Pharmacokinetics

Treatment of Primary Immunodeficiency

In the clinical study assessing the efficacy and safety of Privigen™ in 80 subjects with PI (*see Clinical Studies [14.1]*), serum concentrations of total IgG and IgG subclasses were measured in 25 subjects (ages 13 to 69) following the 7th infusion for the 3 subjects on the 3-week dosing interval and following the 5th infusion for the 22 subjects on the 4-week dosing

interval. After the infusion, blood samples were taken until Day 21 and Day 28 for the 3-week and 4-week dosing intervals, respectively.

Table 4 summarizes the pharmacokinetic parameters of Privigen™, measured as serum concentrations of total IgG.

Table 4: Pharmacokinetic Parameters of Privigen™ in Subjects with PI

Parameter	3-Week Dosing Interval (n=3)		4-Week Dosing Interval (n=22)	
	Mean (SD)	Median (Range)	Mean (SD)	Median (Range)
C_{max} (peak, mg/dL)	2,550 (400)	2,340 (2,290-3,010)	2,260 (530)	2,340 (1,040-3,460)
C_{min} (trough, mg/dL)	1,230 (230)	1,200 (1,020-1,470)	1,000 (200)	1,000 (580-1,360)
$t_{½}$ (days)	27.6 (5.9)	27.8 (21.6-33.4)	45.4 (18.5)	37.3 (20.6-96.6)
AUC_{0-t} (day × mg/dL)*	32,820 (6,260)	29,860 (28,580-40,010)	36,390 (5,950)	36,670 (19,680-44,340)
Clearance (mL/day/kg)*	1.3 (0.1)	1.3 (1.1-1.4)	1.3 (0.3)	1.3 (0.9-2.1)

C_{max}, maximum serum concentration; C_{min}, trough (minimum level) serum concentration; $t_{½}$, elimination half-life; AUC_{0-t}, area under the curve from 0 hour to last sampling time.
* Calculated by log-linear trapezoidal rule.

The median half-life of Privigen™ was 36.6 days for the 25 subjects in the pharmacokinetic subgroup.

Although no systematic study was conducted to evaluate the effect of gender and age on the pharmacokinetics of Privigen™, based on the small sample size (11 males and 14 females) it appears that clearance of Privigen™ is comparable between males (1.27 ± 0.35 mL/day/kg) and females (1.34 ± 0.22 mL/day/kg). In six subjects between 13 and 15 years of age, the clearance of Privigen™ (1.35 ± 0.44 mL/day/kg) is comparable to that observed in 19 adult subjects 19 years of age or older (1.29 ± 0.22 mL/day/kg).

The IgG subclass levels observed in the pharmacokinetic study were consistent with a physiologic distribution pattern (mean trough values): IgG_1, 564.91 mg/dL; IgG_2, 394.15 mg/dL; IgG_3, 30.16 mg/dL; IgG_4, 10.88 mg/dL.

Treatment of Chronic Immune Thrombocytopenic Purpura

Pharmacokinetic studies with Privigen™ were not performed in subjects with chronic ITP.

14 CLINICAL STUDIES

14.1 Treatment of Primary Immunodeficiency

A prospective, open-label, single-arm, multicenter study assessed the efficacy, safety, and pharmacokinetics of Privigen™ in adult and pediatric subjects with PI, who were treated for 12 months at a 3-week or 4-week dosing interval. Subjects ranged in age from 3 to 69; 57.5% were female and 42.5% were male; 77.5% were Caucasian, 15% were Hispanic, and 7.5% were African-American. All subjects had been on regular IGIV replacement therapy for at least 6 months prior to participating in the study.

The efficacy analysis included 80 subjects, 16 on the 3-week dosing interval and 64 on the 4-week dosing interval. Doses ranged from 200 mg/kg to 888 mg/kg. The median dose for the 3-week interval was 428.3 mg/kg; the median dose for the 4-week interval was 440.6 mg/kg. Subjects received a total of 1038 infusions of Privigen™, 272 in the 3-week dosing interval and 766 in the 4-week dosing interval. The maximum infusion rate allowed during this study was 8 mg/kg/min with 69% (715) of the infusions administered at a rate of 7 mg/kg/min or greater.

The primary endpoint was the annual rate of acute serious bacterial infections (aSBIs), defined as pneumonia, bacteremia/septicemia, osteomyelitis/septic arthritis, bacterial meningitis, and visceral abscess, per subject per year. Secondary endpoints included days out of work/school/day care or days unable to perform normal activities due to illness, days of hospitalization, and use of antibiotics.

During the 12-month study period, the aSBI rate was 0.08 (with an upper 1-sided 99% confidence interval of 0.203), which met the predefined success rate of less than one aSBI per subject per year. Six subjects experienced an aSBI, including three cases of pneumonia and one case each of septic arthritis, osteomyelitis, and visceral abscess. All six subjects completed the study.

The rate of other infections was 3.55 infections per subject per year. The infections that occurred most frequently were sinusitis (31.3%), nasopharyngitis (22.5%), upper respiratory tract infection (18.8%), bronchitis (13.8%), and rhinitis (13.8%). The majority of these infections were mild or moderate; among the 255 infections, 16 (6.3%) occurring in 10 subjects were considered severe.

Table 5 summarizes the efficacy results for all 80 subjects.

Table 5: Summary of Efficacy Results in Subjects With PI

Number of Subjects	80
Results from Case Report Forms	
Total Number of Subject Days	26,198
Infections Annual rate of confirmed aSBIs* Annual rate of other infections	 0.08 aSBIs/subject year† 3.55 infections/subject year
Antibiotic use Number of subjects (%) Annual rate	 64 (80%) 87.4 days/subject year
Results from Subject Diaries	
Total Number of Diary Days	24,059
Out of work/school/ day care or unable to perform normal activities due to illness Number of days (%) Annual rate	 570 (2.37%) 8.65 days/subject year
Hospitalization Number of days (%) Annual rate	 166 (0.69%) 2.52 days/subject year

* Defined as pneumonia, bacterial meningitis, bacteremia/septicemia, osteomyelitis/septic arthritis, and visceral abscess.

† Upper 1-sided 99% confidence interval: 0.203.

14.2 Treatment of Chronic Immune Thrombocytopenic Purpura

A prospective, open-label, single-arm, multicenter study assessed the efficacy, safety, and tolerability of Privigen™ in 57 subjects with chronic ITP and a platelet count of 20 x 10^9/L or less. Subjects ranged in age from 15 to 69; 59.6% were female and 40.4% were male; all were Caucasian.

Subjects received a 2 g/kg dose of Privigen™ administered daily as two 1 g/kg intravenous infusions for 2 consecutive days and were observed for 29 days. Fifty-three (93%) subjects received Privigen™ at the maximum infusion rate allowed (4 mg/kg/min [0.04 mL/kg/min]).

The primary endpoint was the response rate defined as the percentage of subjects with an increase in platelet counts to at least 50 x 10^9/L within 7 days after the first infusion (responders). Secondary endpoints included the increase in platelet counts and the time to reach a platelet count of at least 50 x 10^9/L at any point within the study period, the duration of that response, and the regression (decrease in the severity) of hemorrhage in subjects who had bleeding at baseline. Platelet counts were measured on Days 1, 2, 4, 6, 8, 15, 22, and 29. Additional measurements on Days 57 and 85 occurred in subjects with a platelet count of at least 50 x 10^9/L at the previous visit.

Of the 57 subjects in the efficacy analysis, 46 (80.7%) responded to Privigen™ with a rise in platelet counts to at least 50 x 10^9/L within 7 days after the first infusion. The lower bound of

the 95% confidence interval for the response rate (69.2%) is above the predefined response rate of 50%.

The highest median increase in platelet counts was seen 7 days after the first infusion (123 x 10^9/L). The median maximum platelet count achieved was 154 x 10^9/L. The median time to reach a platelet response of more than 50 x 10^9/L was 2.5 days after the first infusion. Twenty-five (43%) of the 57 subjects reached this response by Day 2 prior to the second infusion and 43 (75%) subjects reached this response by Day 6.

The duration of platelet response was analyzed for the 48 subjects who achieved a response any time after the first infusion. The median duration of platelet response in these subjects was 15.4 days (range: 1 to >82 days). Thirty-six (75%) of the 48 subjects maintained the response for at least 8.8 days and 12 (25%) of them for at least 21.9 days. Five (9%) subjects maintained a response up to Day 29 and two (4%) up to Day 85.

A decrease in the severity of hemorrhage from baseline was observed in the following bleeding locations: skin (31 of 36 subjects), oral cavity (11 of 11 subjects), and genitourinary tract (7 of 9 subjects). This decrease was not sustained in all subjects up to the end of the 29-day study period.

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16 HOW SUPPLIED/STORAGE AND HANDLING

Privigen™ is supplied in a single-use, tamper-evident vial containing the labeled amount of functionally active IgG. The components used in the packaging for Privigen™ are latex-free.

The following dosage forms are available:

NDC Number	Fill Size (mL)	Grams
44206-436-05	50	5
44206-437-10	100	10
44206-438-20	200	20

Each vial has an integral suspension band and a label with two peel-off strips showing the product name, lot number, and expiration date.

When stored at room temperature (up to 25°C [77°F]), Privigen™ is stable for up to 24 months, as indicated by the expiration date printed on the outer carton and vial label. Do not freeze. Keep Privigen™ in its original carton to protect it from light.

17 PATIENT COUNSELING INFORMATION

17.1 Renal Dysfunction

Instruct patients to immediately report symptoms of decreased urine output, sudden weight gain, fluid retention/edema, and/or shortness of breath. Such symptoms may suggest kidney damage (*see Boxed Warning, Warnings and Precautions [5.1]*).

17.2 Aseptic Meningitis Syndrome (AMS)

Instruct patients to immediately report signs and symptoms of AMS. These symptoms include severe headache, neck stiffness, drowsiness, fever, sensitivity to light, painful eye movements, nausea, and vomiting (*see Warnings and Precautions [5.2]*).

17.3 Hemolysis

Instruct patients to immediately report signs and symptoms of hemolysis. These symptoms include fatigue, increased heart rate, yellowing of the skin or eyes, and dark-colored urine (*see Warnings and Precautions [5.3]*).

17.4 Transfusion-Related Acute Lung Injury (TRALI)

Instruct patients to immediately report signs and symptoms of TRALI, which is characterized by severe respiratory distress, pulmonary edema, hypoxemia, normal left ventricular function, and fever. TRALI typically occurs within 1 to 6 hours following transfusion (*see Warnings and Precautions [5.4]*).

17.5 Transmissible Infectious Agents

Inform patients that Privigen™ is made from human plasma (part of the blood) and may contain infectious agents that can cause disease (e.g., viruses, and, theoretically, the CJD agent). Explain that the risk that Privigen™ may transmit an infectious agent has been reduced by screening the plasma donors, by testing the donated plasma for certain virus infections, and by inactivating and/or removing certain viruses during manufacturing (*see Warnings and Precautions [5.6]*).

17.6 Live Virus Vaccines

Inform patients that administration of IgG may transiently impair the effectiveness of live virus vaccines (e.g., measles, mumps, and rubella) and to notify their immunizing physician of recent therapy with Privigen™ (*see Drug Interactions [7.1]*).

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REVIEW ARTICLE

DRUG THERAPY

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THE USE OF INTRAVENOUS IMMUNE GLOBULIN IN IMMUNODEFICIENCY DISEASES

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WITH the discovery of agammaglobulinemia in 1952,[1] a pressing need emerged for antibody-replacement therapy to prevent serious bacterial infections. Intramuscularly administered immune serum globulin resulted in a remarkable decrease in the incidence of such infections in patients with this type of immunodeficiency; however, the injections were painful, and the IgG was absorbed slowly and was subject to local proteolysis. Intravenous administration of immune serum globulin resulted in shock-like episodes, chills, and hyperpyrexia, especially in sick and acutely infected children. Beginning in the early 1960s, many attempts were made to modify immune serum globulin so that it could be given safely intravenously.[2] Intravenous injections were less painful to receive than intramuscular injections, much larger doses could be given, and high serum levels of antibody could be achieved immediately. A number of preparations were used in Europe in the 1960s and 1970s.[3,4] However, immune serum globulin for intramuscular use remained the sole form of replacement therapy in the United States until 1981, when one form for intravenous use became available.[5] Six intravenous preparations of immune globulin have now been approved by the Food and Drug Administration and are available in the United States (Table 1), and nearly three dozen more are under investigation or marketed abroad.[6-8]

PREPARATIONS OF INTRAVENOUS IMMUNE GLOBULIN

Nearly all preparations are isolated initially from normal plasma by the Cohn alcohol fractionation method[9,10] or a modification of it.[11] In this process, serum proteins are separated in the cold by a sequence of precipitations with ethanol at low ionic strength and pH. Although IgG constitutes 95 to 99 percent of the protein in Cohn Fraction II, varying but small quantities of IgA, IgM, IgD, IgE, and other proteins are also present. The IgG in Cohn Fraction II tends to aggregate, which was thought to explain the adverse reactions that occurred when immune serum globulin was administered intravenously. Several different procedures have been developed to counter the tendency of these preparations to aggregate. Cleavage with pepsin prevents IgG aggregation but results in fragments with a very short half-life and decreased function. However, treatment of Cohn Fraction II at low pH with a trace of pepsin or with immobilized pepsinogen results in fully functional, well-tolerated preparations of intravenous immune globulin. The first intravenous immune globulin marketed in the United States was prepared by modifying the Fc portion of the molecule by reduction and alkylation, but such treatment altered the Fc function of the IgG, as did sulfonation and treatment with propiolactone. This preparation was replaced in 1987 with one prepared by treatment at pH 4.25, which resulted in fully functional intact IgG molecules that are well tolerated. Polyethylene glycol and DEAE–Sephadex or ethanol at low ionic strength have also been used successfully to remove aggregates without altering IgG Fc function. Additional stabilizing agents, such as various sugars, amino acids (glycine), and albumin, are added to prevent reaggregation and to protect the IgG molecule during lyophilization.

In 1982 a committee of the World Health Organization[12] established the following criteria for the production of intravenous immune globulin: each lot should be derived from plasma pooled from at least 1000 donors; it should be free of prekallikrein activator, kinins, plasmin, and preservatives; it should contain at least 90 percent intact IgG, with the subclasses present in normal ratios, and be as free as possible of aggregates; its IgG molecules should maintain all of their biologic activity, such as the ability to fix complement; and the preparations should be free of infectious agents and "other potentially harmful contaminants." In practice, all plasma is screened for hepatitis B surface antigen, human immunodeficiency virus (HIV), and elevated levels of alanine aminotransferase and soon will be screened for antibodies to hepatitis C and hepatitis B core antigen. All commercial lots are produced from plasma pooled from 3000 to 6000 donors and therefore contain a broad spectrum of antibodies. Each pool must contain adequate levels of antibody to antigens in various vaccines, such as tetanus and measles. However, there is no standardization based on titers of antibodies to more clinically relevant organisms, such as *Streptococcus pneumoniae* or *Haemophilus influenzae*. Although there is some variation in antibody titers between preparations from the various manufacturers,[13,14] as well as from lot to lot with each preparation, there is no evidence that there

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Table 1. Characteristics of the Preparations of Intravenous Immune Globulin Available in the United States.

Brand Name	Manufacturing Process	Additives	Approximate IgA Content* (μg/ml)	Form Supplied	Manufacturer
Gamimune N	pH 4.25, diafiltration	10% maltose	270	5% liquid, pH 4.25	Cutter Biological, Miles Laboratories
Gammagard†	Polyethylene glycol, DEAE–Sephadex, ultrafiltration	2% maltose, 0.2% polyethylene glycol, 0.3 M glycine, 0.15 M sodium chloride, 3% albumin	0.4–1.9‡	Lyophilized, 5%, pH 6.8	Hyland Division, Baxter Healthcare
Gammar-IV	Low-ionic-strength ethanol	5% sucrose, 2.5% albumin, 0.5% sodium chloride	20‡	Lyophilized, 5%, pH 7.0	Armour Pharmaceutical
IVEEGAM	Immobilized trypsin, polyethylene glycol	5% glucose, 0.3% sodium chloride, 0.5 polyethylene glycol	5‡	Lyophilized, 5%, pH 6.8	Immuno-US
Sandoglobulin	pH 4.0, 1:10,000 trypsin	5% or 10% sucrose (sodium chloride in diluent)	720	Lyophilized, 3% or 6%, pH 6.6	Sandoz Pharmaceutical
Venoglobulin-I	Polyethylene glycol, DEAE–Sephadex	2% D-mannitol, 1% albumin, 0.5% sodium chloride, <0.6% polyethylene glycol	24‡	Lyophilized, 5%, pH 6.8	Alpha Therapeutics

*Some data were obtained from Römer et al.[4] and Apfelzweig et al.[17] Values are approximate: there is a great deal of lot-to-lot variability.
†Another preparation, marketed by the American Red Cross, is prepared by Baxter Hyland with plasma from Red Cross volunteer donors.
‡Data provided by manufacturer.

are consistent differences in antibody titers between the preparations. Many assays of antibody potency do not correlate with biologic activity[14]; therefore, standardization of methods will be necessary before it will be possible to make valid comparisons of antibody titers in various preparations of intravenous immune globulin.

Not surprisingly, preparations of immune globulin containing high titers of specific antibody have proved to be more effective than standard preparations for the prevention of infection with agents such as group B streptococci[15] and cytomegalovirus.[14] Eventually, preparations containing high titers of antibodies to specific organisms will be available, but the logistic problems in producing these are enormous. Removal of HIV-positive plasma from the pool has already eliminated a donor population with high titers of antibodies to many infectious agents, including cytomegalovirus, herpes simplex virus, and pneumococci. Intensive research is being devoted to the development of pools of monoclonal antibodies derived from human hybridomas that can be used alone or in combination to prevent or treat specific infections. One such human monoclonal antibody against endotoxin has already been shown to be safe and effective in a randomized, double-blind trial in patients with sepsis.[16]

The characteristics of the six preparations of intravenous immune globulin approved for use in the United States are summarized in Table 1. A seventh preparation, marketed by the American Red Cross, is prepared by Baxter Hyland Therapeutics with plasma from Red Cross volunteer donors and is functionally identical to Gammagard (Baxter Hyland). The properties of several of these preparations have been reviewed.[3,4,6-8,18-20] All are well tolerated and effective in most patients with humoral immunodeficiency.

Clinical Use in Immunodeficiency

Indications

Primary Immunodeficiency

Immune globulin replacement therapy should always be undertaken with the realization that the goal is to provide IgG antibodies — not a certain number of milligrams of IgG — to those who lack them. Thus, the only clear indication for replacement therapy with immune globulin is severe impairment in antibody-forming capacity.[21] Such defects are found in primary immunodeficiency diseases characterized by low or undetectable quantities of all five serum immunoglobulin classes, such as X-linked agammaglobulinemia, common variable immunodeficiency, and severe combined immunodeficiency, as well as X-linked immunodeficiency with hyperimmunoglobulinemia M. In addition, antibody therapy is indicated for patients with normal or near-normal immunoglobulin levels who have a marked impairment in their ability to produce specific antibodies after immunization. The latter include boys with the Wiskott–Aldrich syndrome, who are unable to produce antibodies to polysaccharide antigens and have blunted anamnestic responses to protein antigens, and patients with ataxia telangiectasia,[21] but may also include patients who have neither of these syndromes.[22,23]

In contrast, most children with transient hypogammaglobulinemia of infancy make normal amounts of specific antibodies in response to immunization, despite low serum concentrations of all or some im-

munoglobulins during the first few months or years of life.[24] The administration of exogenous antibody could inhibit endogenous antibody formation in these patients. It is important to evaluate each patient's ability to make specific antibodies to protein and polysaccharide antigens, but it must be kept in mind that titers of antipolysaccharide antibodies are normally low until after two years of age. The use of intravenous immune globulin should be reserved for patients with serious defects of antibody formation, rather than low immunoglobulin concentrations.

The incidence of sinopulmonary and other infections may be increased in patients with deficiencies of an IgG subclass.[25-29] Concern was heightened when antibodies to carbohydrate antigens were found to be predominantly in the IgG2 subclass.[30] However, IgG1 anticarbohydrate antibody responses may occur in patients with IgG2 deficiency,[29,30] and some healthy children have deficiencies of IgG2.[31] Indeed, deletions of a number of immunoglobulin heavy-chain genes have been reported in humans.[32] Fifteen of the 16 cases of multigene deletions were in healthy people, even though those affected lacked several immunoglobulin subclasses and even one class. The so-called normal concentrations of IgG subclasses vary greatly from laboratory to laboratory, and many apparent subclass deficiencies are laboratory artifacts. It is essential to point out again that it is the presence of impaired antibody responses, not a deficiency of an IgG subclass protein, that is associated with an increased incidence of infection and a beneficial response to intravenous immune globulin.[25] Thus, the only clear indication for replacement therapy with intravenous immune globulin in patients with selective IgG subclass deficiency is a demonstrated deficiency in the ability to form antibodies against a variety of polysaccharide and protein antigens.

Secondary Immunodeficiencies

Abnormalities of serum immunoglobulin levels or of antibody formation may develop as a result of a variety of other diseases or trauma.[33] Antibody deficiency may occur in previously normal persons who have hematologic cancers[34] or who receive immunosuppressive agents[35] for the treatment of cancer or in anticipation of bone marrow transplantation. The efficacy of intravenous immune globulin in patients with chronic lymphocytic leukemia[36] was demonstrated in a large multicenter, placebo-controlled study.[37] The use of intravenous immune globulin after bone marrow transplantation reduced the incidence and severity of interstitial pneumonia related to cytomegalovirus, sepsis, and graft-versus-host disease.[38] Serum IgG concentrations may be quite low in protein-losing states, such as the nephrotic syndrome, intestinal lymphangiectasia, protein-losing enteropathy, burns, or after repeated plasmapheresis. In most patients with such disorders, IgG antibody formation is normal; thus, there is little evidence that intravenous immune globulin would be beneficial unless there is an ongoing active infection. Even then, the infused intravenous immune globulin would be lost by the same route as the native IgG molecules. Patients with HIV infection, particularly infants, may have profound defects in their ability to form specific antibodies, despite the fact that their serum immunoglobulin levels are usually elevated.[39] In small trials in such patients intravenous immune globulin reduced the incidence of pyogenic infections and prolonged survival,[40] reduced serum lactate dehydrogenase levels,[41] and reduced the levels of suppressor T cells,[42] even though it did not alter the ultimate mortality rate. In a recent multicenter, randomized, double-blind, placebo (albumin)-controlled trial, described in this issue of the *Journal*, in 372 HIV-infected children without hemophilia who were less than 13 years of age, intravenous immune globulin, in a dose of 400 mg per kilogram of body weight per month, was effective in prolonging infection-free time in immunologically impaired or clinically symptomatic children with CD4 cell counts of 0.2×10^9 per liter or above.[43]

Contraindications

IgA deficiency is the most common of the well-defined primary immunodeficiency disorders,[21,44] and many patients have chronic or recurrent infections of the mucous membranes.[21] Some patients with selective IgA deficiency and IgG subclass deficiencies have impaired antibody responses to carbohydrate antigens.[29] However, most patients with selective IgA deficiency produce normal quantities of IgG antibodies of all types. Secretory IgA in normal persons is produced in lymphoid tissue located near the gut and respiratory tract and is not transported from the intravascular compartment for external secretion. Even if products containing IgA could be given safely, they would not effect replacement of that immunoglobulin at mucous-membrane surfaces, since radiolabeled IgA given intravenously to normal subjects could not be detected in saliva or breast milk.[45]

Approximately 40 percent of patients with selective IgA deficiency produce antibodies to IgA,[49] and these antibodies have been implicated as the cause of anaphylactic transfusion reactions in some patients.[46-48] Similarly, serum IgA is often undetectable in patients with common variable immunodeficiency, and anti-IgA antibodies have been detected in a number of such patients.[49,50] Thus, therapy with intravenous immune globulin is not indicated in patients with selective IgA deficiency, and caution should be exercised during its administration to patients with common variable immunodeficiency, particularly those with no detectable IgA.

Metabolism

Radiolabeled IgG is eliminated according to multicompartmental first-order kinetics[49,51]; that is, the elimination is dependent on the serum concentration. This has been substantiated in studies in which large

quantities of IgG were infused into patients with immunodeficiency.[52-59] Approximately half the IgG is redistributed to the extravascular compartment during the first three to five days after intravenous infusion.[51] The half-life of IgG is 18 to 23 days in normal persons, but this varies among the subclasses. IgG1, IgG2, and IgG4 all have half-lives close to that range, but IgG3 is eliminated more rapidly, with a half-life in normal persons of 7.5 to 9 days.[51,55]

The half-life of IgG is highly variable among patients with hypogammaglobulinemia, but is usually longer than in normal persons and can exceed 70 days.[52,56] With prolonged therapy with intravenous immune globulin, the half-life may become longer as a result of a decrease in the extent of infection,[57] because less antibody is lost by binding to infectious agents in the patient.

Dosage

Until the 1980s, the recommended dose of intramuscular immune serum globulin for the treatment of severe primary humoral immunodeficiency was 100 mg per kilogram each month, after a loading dose that was two to three times that amount. The doses were selected empirically[58-61] but were determined in part by the maximal volume that could be delivered intramuscularly. The mean trough serum IgG concentration in patients with agammaglobulinemia four weeks after the administration of 100 mg of immune serum globulin per kilogram was usually less than 100 mg per deciliter and not more than 150 mg per deciliter.[62]

The optimal dose of IgG for antibody replacement in patients with primary disorders of humoral immunity has not been studied prospectively. The only prospective clinical trial comparing intramuscular immune serum globulin with intravenous immune globulin did not show a difference four weeks after treatment in trough serum IgG concentrations when both were administered in doses of 100 mg per kilogram each month.[5] A study comparing the clinical effects of monthly doses of 100 mg per kilogram and 400 mg per kilogram of a reduced and alkylated preparation of intravenous immune globulin found no difference in the number of days of illness or in the need for antibiotics, although the patients subjectively felt better when given the higher dose.[63] In patients given a dose of 100 mg per kilogram each month, however, the titers of specific antibodies were often undetectable days to weeks before the next infusion, indicating a risk of serious infection.[52] Several other prospective studies compared the effects in patients receiving doses of 100 or 250 mg per kilogram,[64] 150 or 500 mg per kilogram,[65] or 200 or 600 mg per kilogram[65-68]; all reported improvement in symptom scores at the higher doses. In addition, the recipients of the higher doses had much higher trough serum IgG concentrations and specific antibody titers. Although none of these studies were blinded, the overall consensus is that monthly doses of 300 to 400 mg per kilogram are superior to lower doses for most patients.

Because of differences in the half-life of IgG in individual patients,[52,56,62,69] serum IgG should be measured before each infusion, and the dose of intravenous immune globulin adjusted accordingly. Trough serum IgG concentrations four weeks after treatment should be maintained at 400 to 500 mg per deciliter, a value close to the lower limit of normal. When ongoing infection results in a more rapid rate of catabolism of antibodies to the particular infectious agent, higher doses must be given at more frequent intervals. The best examples of this are acute bacterial infections in patients with any type of antibody deficiency and persistent enterovirus infections of the central nervous system in patients with X-linked agammaglobulinemia.[70] In the latter patients, very high doses of intravenous immune globulin are needed, and intrathecal administration of immune globulin through an Ommaya reservoir has proved beneficial.[71]

Infusions are usually given every four weeks, but the interval should be adjusted, depending on the trough serum IgG concentration and the patient's clinical condition. Longer intervals may be possible if higher doses are given[65]; conversely, more frequent infusions are required in patients with rapid catabolism of IgG or ongoing infection.

Administration

The recommended rates of infusion of intravenous immune globulin were determined empirically in early studies using reduced and alkylated IgG without added sugar.[5] Such preparations led to rate-related adverse reactions in nearly 50 percent of the patients. Even though the newer preparations are tolerated much better, manufacturers have continued to recommend the rates used in the initial studies. These rates are 0.01 to 0.02 ml per kilogram per minute initially, with increases of up to 0.1 ml per kilogram per minute. This results in a maximal rate of approximately 5 mg per kilogram per minute. However, higher rates can be tolerated by some patients with immunodeficiency diseases. In a recent study, patients were infused with 6 to 12 percent IgG at rates of up to 30 mg per kilogram per minute.[72] Thirteen of the 16 patients completed the study, and no serious adverse reactions occurred. Many patients received their infusions in less than 20 minutes, which greatly reduced the inconvenience of therapy. The patients were selected for this study because they had not had any adverse reactions at the standard rates of infusion; therefore, caution must be exercised in attempting to increase the infusion rate in any individual patient, especially early in therapy, when the risk of adverse reactions is highest. Further studies using other preparations of intravenous immune globulin are needed to establish new guidelines for infusion rates in patients with immunodeficiency diseases. Home administration has been used by some to reduce the inconvenience of this ther-

apy.[73,74] Although few adverse reactions were noted in the carefully selected patients who were treated at home, serious reactions may occur, and in our opinion, the risks outweigh the small benefits for most patients.

Costs of Therapy

The cost of therapy with intravenous immune globulin is high. The average wholesale prices of the different preparations available in the United States range from $35 to $65 per gram. The prices paid by consumers may be roughly twice these amounts. In an informal survey we found that the charges ranged from $125 to $250 per infusion. Thus, the annual costs of administering intravenous immune globulin at a dose of 400 mg per kilogram per month to a 10-kg infant range from $3,180 to $4,620 wholesale and $4,860 to $8,220 retail; annual costs for a 70-kg adult would be $13,260 to $23,340 wholesale and $25,020 to $45,180 retail.

Adverse Reactions

Nonanaphylactic Reactions

Nonanaphylactic reactions are the most common type of reaction to intravenous immune globulin.[3-5,75] They are characterized by back or abdominal pain, nausea, and often vomiting, within the first 30 minutes of the infusion. Usually there is no dyspnea, hypotension, or other change in vital signs. Chills, fever, headache, myalgia, and fatigue may begin at the end of the infusion and continue for several hours.[5,75,76] Most of these reactions can be prevented by slowing the initial rate of infusion or by pretreatment with aspirin. Interrupting the infusion for a few minutes will often allow this type of reaction to subside, and when the infusion is resumed, the rate can usually be increased without much difficulty. More severe reactions of this type may require pretreatment with corticosteroids.[77,78] These reactions may be caused by the binding of the infused antibodies to the antigens of infectious agents in the patient. The frequency and severity of this type of reaction diminish after acute or chronic bacterial infections, particularly of the lungs, have been controlled with antibiotic therapy and regular administration of adequate doses of intravenous immune globulin.

Anaphylactic Reactions

True anaphylactic reactions to intravenous immune globulin are rare. Such reactions have been noted in patients with selective IgA deficiency or common variable immunodeficiency who had antibodies to IgA after treatment with immune serum globulin or intravenous immune globulin.[46,47,49,50] The symptoms were those of classic IgE-mediated anaphylactic reactions: flushing, facial swelling, dyspnea, cyanosis, anxiety, nausea, vomiting, malaise, hypotension, loss of consciousness, and in some cases, death. They appeared seconds to minutes to several hours after the infusion. We evaluated two patients with common variable immunodeficiency who had anaphylactic reactions and found that both had high titers of IgE and IgG antibodies to IgA, despite very low total serum IgE and IgG concentrations.[50] However, these increases in the titers of IgE antibodies to IgA correlated more closely with clinical reactions to intravenous immune globulin than did increases in the concentration of IgG antibodies to IgA. The treatment of such reactions is immediate discontinuation of intravenous immune globulin and administration of epinephrine, oxygen, antihistamines, and intravenous steroids. Gammagard, which contains very low concentrations of IgA,[17,50] has been well tolerated by several of these patients. However, each lot of every brand of immune globulin, even Gammagard, must be screened to ensure that it contains less than 1.5 μg of IgA per milliliter, and all infusions should be given under carefully controlled circumstances.

Transmission of Infectious Agents

Transmission of viral agents, particularly hepatitis B, by intramuscular immune serum globulin was occasionally reported before the institution of donor screening.[79] Fortunately, the cold-ethanol fractionation process substantially reduces the amount of hepatitis B virus present in plasma products.[80] It is also fortuitous that ethanol inactivates HIV type 1 (HIV-1) since, in the early 1980s, intravenous immune globulin was prepared unintentionally from the plasma of HIV-infected donors and the recipients had transiently positive tests for antibodies to HIV. However, there have been no reports of transmission of HIV infection by intravenous immune globulin. Experiments in which HIV-1 was added to plasma before Cohn fractionation demonstrated that the procedure reduced the level of HIV-1 to 10^{-15} of the initial dose.[8]

Hepatitis C has been reported in a total of 19 patients receiving investigational preparations of intravenous immune globulin in the United States[82] and England[83] and in some patients receiving commercially available preparations in Scotland[84] and Sweden.[85,86] The ability to screen for hepatitis C should help avoid this problem in the future. A retrospective survey of serum aminotransferase levels in large numbers of patients treated with intravenous immune globulin prepared at pH 4.2 (Gamimune N) in the United States did not reveal any evidence of hepatitis in these patients,[87] nor did a similar survey in Australia.[88] In our 16 years of experience with the administration of multiple lots of six types of intravenous immune globulin in more than 75 patients with immunodeficiency who were treated monthly, no patient has had a substantial increase in serum aminotransferase values.

Viruses in plasma fractions can be inactivated by propiolactone, polysorbate 80 (Tween 80) and ether,[89] tributyl phosphate and cholate,[90] or polyethylene glycol at low ionic strength.[91] Even prolonged incubation at room temperature greatly reduces the risk of infectivity. It is likely that the next generation of prepara-

tions of intravenous immune globulin will include a viral-inactivation step. Until then, potential transmission of some viral agents must be considered a small but definite risk when a decision is made to institute therapy.[92]

Potential Risks in Persons Capable of Antibody Production

Although both intramuscular immune serum globulin and intravenous immune globulin have seemingly proved quite safe when administered to large numbers of both normal persons and persons with immunodeficiency diseases, the fact that intravenous immune globulin has known immunomodulating properties[93] should be kept in mind when one is considering its use in persons without antibody deficiency. Passively administered antibodies are potent antigen-specific immunosuppressive agents, as attested by the high degree of efficacy of $Rh_o(D)$ immune globulin (RhoGAM) in preventing sensitization of Rh-negative women to the Rh D antigen on the red cells of their Rh-positive fetuses. Recent studies in patients with systemic vasculitis demonstrated a 51 percent decrease in antineutrophil cytoplasm antibodies after high-dose therapy with intravenous immune globulin, and this decrease was maintained during follow-up.[94] Although there are no controlled clinical studies of the suppression of immune responses to other antigens by passively administered antibody, there is no reason to believe that antibodies to the Rh D and neutrophil cytoplasm antigens are unique in this regard. There is also clear evidence from animal and in vitro studies that intravenous immune globulin can suppress antibody formation,[95-97] T-cell proliferation,[98] and the activity of natural killer cells.[99] In addition to the masking of antigens, intravenous immune globulin has many potential mechanisms for suppressing the immune response. These include interaction with Fc receptors on the membranes of various cells of the immune system and the combination of antiidiotypic antibodies with either antibody-producing cells or secreted antibodies.[94,100] Antiidiotypic antibodies in intravenous immune globulin could theoretically impair the capacity of B cells from even immune hosts to secrete antibody. Finally, blockade of the Fc receptor by high doses of intravenous immune globulin (as in the treatment of idiopathic thrombocytopenic purpura[100]) or by the formation of immune complexes of intravenous immune globulin with antigens could also impair the normal clearance of opsonized infectious agents and lead to overwhelming infection. Thus, there are many reasons not to administer intravenous immune globulin unless there is a demonstrated broad antibody-deficiency state or other accepted clinical indications.

Conclusions

The development of safe and effective intravenous preparations of immune globulin represents a major advance in the treatment of patients with severe antibody deficiencies, although such therapy is expensive. Broad antibody deficiency should be carefully documented before therapy is initiated. The rationale for the use of these preparations is to provide missing antibodies, not to raise the serum IgG concentration. In general, the preparations currently available in the United States have similar antibody titers and are equally effective and safe. The one exception is that Gammagard has very low levels of IgA, and carefully screened lots of it can be used safely in patients who have antibodies to IgA. There has been no documented transmission of HIV infection or hepatitis by any of these preparations.

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ibpn international blood/plasma news

* **TALECRIS BIOTHERAPEUTICS reports that in the first quarter of 2010 it was able to source 69% of its plasma requirements for manufacturing from its own platform of collection centers.** The balance was supplied by third parties, prominently including **CSL PLASMA**, a U.S. operating unit of **CSL BEHRING**. Over the next several years, Talecris plans to increase the volume of plasma generated by its own centers to meet over 90% of overall needs, according to a senior official in a conference call to report company earnings.

Talecris has notified CSL Plasma that it will not elect its option to purchase any plasma in 2011, noting that its incremental cost of production is well below the average price per liter in that contract. Talecris plans instead to increase throughput by its own 69 plasma collection centers, a strategy it says "will drive operating efficiency and improve our internal cost per liter."

* **CSL BEHRING announced that the U.S. FDA has approved a supplemental Biologics License Application (sBLA) that extends the shelf life of its *Privigen* 10% liquid intravenous immunoglobulin product from 24 months to 36 months.** This approval makes *Privigen* the first liquid IVIG in the U.S. that can be stored at room temperature (up to 25°C [77°F]) throughout its entire 36-month shelf life.

Privigen is stabilized with proline, a naturally occurring amino acid. Use of proline at pH 4.8 minimizes dimer formation (under a specification of ≤12% dimers) and is the key to allowing the product to be stored at room temperature for up to 36 months. In a three-year study conducted by CSL Behring to assess the product's physicochemical, biological and immunological parameters, *Privigen* maintained high purity (≥98% IgG), with no formation of aggregated IgG molecules.

HIGHLIGHTS OF PRESCRIBING INFORMATION

These highlights do not include all the information needed to use Hizentra safely and effectively. See full prescribing information for Hizentra.

Hizentra, Immune Globulin Subcutaneous (Human), 20% Liquid
Initial U.S. Approval: 2010

----------INDICATIONS AND USAGE----------

Hizentra is an Immune Globulin Subcutaneous (Human) (IGSC), 20% Liquid indicated for the treatment of primary immunodeficiency (PI) (1).

----------DOSAGE AND ADMINISTRATION----------

For subcutaneous infusion only. Do not inject into a blood vessel.
Begin treatment with Hizentra one week after the patient's last Immune Globulin Intravenous (Human) (IGIV) infusion.

Dosage (2.2)

The initial weekly dose of Hizentra is calculated to achieve a systemic serum IgG exposure (area under the concentration-time curve [AUC]) not inferior to that of the previous IGIV treatment.

- Initial dose = $\frac{1.53 \times \text{previous IGIV dose (in grams)}}{\text{No. of weeks between IGIV doses}}$
  To convert the dose in grams to milliliters (mL), multiply the calculated dose (in grams) by 5.
- Adjust the dose over time based on clinical response and serum IgG trough levels. Measure the serum IgG trough level after 2 to 3 months of treatment with Hizentra. Adjust the dose to achieve serum IgG trough levels that are 1.3 times the trough level prior to the last IGIV treatment.

Administration (2.3)

- Infusion sites – Abdomen, thigh, upper arm, and/or lateral hip. Use up to 4 injection sites simultaneously, with at least 2 inches between sites.
- Infusion volume – For the first infusion, up to 15 mL per injection site. This may be increased to 20 mL per site after the fourth infusion and to a maximum of 25 mL per site as tolerated.
- Infusion rate – For the first infusion, up to 15 mL/hr per site. This may be increased, to a maximum of 25 mL/hr per site as tolerated. **However, the maximum flow rate is not to exceed a total of 50 mL/hr for all sites combined.**

----------DOSAGE FORMS AND STRENGTHS----------

0.2 g/mL (20%) protein solution for subcutaneous injection (3)

----------CONTRAINDICATIONS----------

- Anaphylactic or severe systemic reactions to human immune globulin or components of Hizentra, such as polysorbate 80 (4)
- Hyperprolinemia (Hizentra contains the stabilizer L-proline) (4)
- IgA-deficient patients with antibodies against IgA and a history of hypersensitivity (4)

----------WARNINGS AND PRECAUTIONS----------

- IgA-deficient patients with anti-IgA antibodies are at greater risk of severe hypersensitivity and anaphylactic reactions. Discontinue use if hypersensitivity reaction occurs (5.1).
- Monitor patients for reactions reported to occur with IGIV treatment that may occur with Hizentra, including renal dysfunction/failure, thrombotic events, aseptic meningitis syndrome (AMS), hemolysis, and transfusion-related acute lung injury (TRALI) (5.2).
- Products made from human plasma can contain infectious agents, e.g., viruses and, theoretically, the Creutzfeldt-Jakob disease (CJD) agent (5.3).

----------ADVERSE REACTIONS----------

The most common adverse reactions, observed in ≥5% of study subjects, were local reactions (i.e., swelling, redness, heat, pain, and itching at the injection site), headache, vomiting, pain, and fatigue (6.1).

To report SUSPECTED ADVERSE REACTIONS, contact CSL Behring Pharmacovigilance at 1-866-915-6958 or FDA at 1-800-FDA-1088 or *www.fda.gov/medwatch.*

----------DRUG INTERACTIONS----------

The passive transfer of antibodies may:

- Lead to misinterpretation of the results of serological testing (5.4, 7.2).
- Interfere with the response to live virus vaccines (7.1).

----------USE IN SPECIFIC POPULATIONS----------

- Pregnancy: No human or animal data. Use only if clearly needed (8.1)

See 17 for PATIENT COUNSELING INFORMATION and the accompanying FDA-approved patient labeling.

Issued: February 2010

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FULL PRESCRIBING INFORMATION

1 INDICATIONS AND USAGE

Hizentra is an Immune Globulin Subcutaneous (Human) (IGSC), 20% Liquid indicated as replacement therapy for primary humoral immunodeficiency (PI). This includes, but is not limited to, the humoral immune defect in congenital agammaglobulinemia, common variable immunodeficiency, X-linked agammaglobulinemia, Wiskott-Aldrich syndrome, and severe combined immunodeficiencies.

2 DOSAGE AND ADMINISTRATION

For subcutaneous infusion only. Do not inject into a blood vessel.

2.1 Preparation and Handling

Hizentra is a clear and pale yellow to light brown solution. Do not use if the solution is cloudy or contains particulates.

- Prior to administration, visually inspect each vial of Hizentra for particulate matter or discoloration, whenever the solution and container permit.
- Do not freeze. Do not use any solution that has been frozen.
- Check the product expiration date on the vial label. Do not use beyond the expiration date.
- Do not mix Hizentra with other products.
- Do not shake the Hizentra vial.
- Use aseptic technique when preparing and administering Hizentra.
- The Hizentra vial is for single-use only. Discard all used administration supplies and any unused product immediately after each infusion in accordance with local requirements.

2.2 Dosage

The dose should be individualized based on the patient's clinical response to Hizentra therapy and serum immunoglobulin G (IgG) trough levels.

Begin treatment with Hizentra one week after the patient's last Immune Globulin Intravenous (Human) (IGIV) infusion. Prior to switching treatment from IGIV to Hizentra, obtain the patient's serum IgG trough level to guide subsequent dose adjustments (*see below under Dose Adjustment*).

Establish the initial weekly dose of Hizentra by converting the monthly IGIV dose into a weekly equivalent and increasing it using a dose adjustment factor. The goal is to achieve a systemic serum IgG exposure (area under the concentration-time curve [AUC]) not inferior to that of the previous IGIV treatment (*see Pharmacokinetics [12.3]*).

Initial Weekly Dose

To calculate the initial weekly dose of Hizentra, multiply the previous IGIV dose in grams by the dose adjustment factor of 1.53; then divide this by the number of weeks between doses during the patient's IGIV treatment (i.e., 3 or 4).

$$\text{Initial Hizentra dose} = \frac{1.53 \times \text{previous IGIV dose (in grams)}}{\text{Number of weeks between IGIV doses}}$$

To convert the Hizentra dose (in grams) to milliliters (mL), multiply the calculated dose (in grams) by 5.

Dose Adjustment

Over time, the dose may need to be adjusted to achieve the desired clinical response and serum IgG trough level. To determine if a dose adjustment may be considered, measure the patient's serum IgG trough level 2 to 3 months after switching from IGIV to Hizentra. The target serum IgG trough level on weekly Hizentra treatment is projected to be 1.3 ± 0.2 times (i.e., between 1.1 and 1.5 times) the last IGIV trough level (*see Pharmacokinetics [12.3]*).

To adjust the dose based on trough levels, calculate the difference (in mg/dL) of the patient's serum IgG trough level from the target IgG trough level (1.3 times the last IGIV trough level). Then find this difference in Table 1 and the corresponding amount (in mL) by which to increase or decrease the weekly dose based on the patient's body weight. **However, the patient's clinical response should be the primary consideration in dose adjustment**.

Table 1: Adjustment (±mL) of the Weekly Hizentra Dose Based on the Difference (±mg/dL) From the Target Serum IgG Trough Level

Difference From Target IgG Trough Level (mg/dL)	Body Weight (kg)												
	10	15	20	30	40	50	60	70	80	90	100	110	120
	Dose Adjustment (mL per Week)*												
50	0	1	1	1	2	2	3	3	4	4	5	5	6
100	1	1	2	3	4	5	6	7	8	8	9	10	11
150	1	2	3	4	6	7	8	10	11	13	14	15	17
200	2	3	4	6	8	9	11	13	15	17	19	21	23
250	2	4	5	7	9	12	14	16	19	21	23	26	28
300	3	4	6	8	11	14	17	20	23	25	28	31	34
350	3	5	7	10	13	16	20	23	26	30	33	36	39
400	4	6	8	11	15	19	23	26	30	34	38	41	45
450	4	6	8	13	17	21	25	30	34	38	42	46	51
500	5	7	9	14	19	23	28	33	38	42	47	52	56

* Dose adjustment in mL is based on the slope of the serum IgG trough level response to Hizentra dose increments (5.3 mg/dL per increment of 1 mg/kg per week).

For example, if a patient with a body weight of 70 kg has an actual IgG trough level of 900 mg/dL and the target trough level is 1000 mg/dL, this results in a difference of 100 mg/dL. Therefore, increase the weekly dose of Hizentra by 7 mL.

Monitor the patient's clinical response, and repeat the dose adjustment as needed.

Dosage requirements for patients switching to Hizentra from another IGSC product have not been studied. If a patient on Hizentra does not maintain an adequate clinical response or a serum IgG trough level equivalent to that of the previous IGSC treatment, the physician may want to adjust the dose. For such patients, Table 1 also provides guidance for dose adjustment if their desired IGSC trough level is known.

Measles Exposure

If a patient is at risk of measles exposure (i.e., due to an outbreak in the US or travel to endemic areas outside of the US), the weekly Hizentra dose should be a minimum of 200 mg/kg body weight for two consecutive weeks. If a patient has been exposed to measles, ensure this minimum dose is administered as soon as possible after exposure.

2.3 Administration

Hizentra is for subcutaneous infusion only. Do not inject into a blood vessel.

Hizentra is intended for weekly subcutaneous administration using an infusion pump. Infuse Hizentra in the abdomen, thigh, upper arm, and/or lateral hip.

- Injection sites – A Hizentra dose may be infused into multiple injection sites. However, do not use more than 4 sites simultaneously. Injection sites should be at least 2 inches apart. Change the actual site of injection with each weekly administration.
- Volume – For the first infusion of Hizentra, do not exceed a volume of 15 mL per injection site. The volume may be increased to 20 mL per site after the fourth infusion and to a maximum of 25 mL per site as tolerated.
- Rate – For the first infusion of Hizentra, the maximum recommended flow rate is 15 mL per hour per site. For subsequent infusions, the flow rate may be increased to a maximum of 25 mL per hour per site as tolerated. **However, the maximum flow rate is not to exceed a total of 50 mL per hour for all sites combined at any time.**

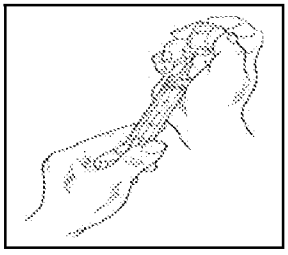
Follow the steps below and use aseptic technique to administer Hizentra.

1. **Assemble supplies** – Place the Hizentra vial(s), disposable supplies (not provided with Hizentra), and other items (infusion pump, patient's treatment diary/log book, sharps or other container) needed for the infusion on a clean, flat surface.

2. **Thoroughly wash and dry hands** – The use of gloves when preparing and administering Hizentra is optional.

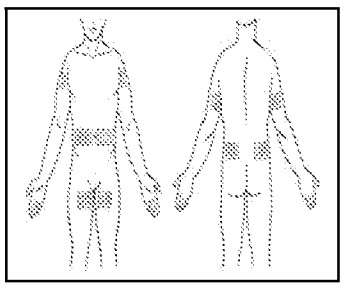
3. **Clean the vial stopper** – Remove the protective cap from the vial to expose the central portion of the rubber stopper. Clean the stopper with an alcohol wipe and allow it to dry.

4. **Prepare and fill the syringe(s)** – Using a sterile syringe and needle, pull back on the plunger to draw air into the syringe that is equal to the amount of Hizentra to be withdrawn. Then, insert the needle into the center of the vial stopper and, to avoid foaming, inject the air into headspace of the vial (not into the liquid). Finally, withdraw the desired volume of Hizentra. When using multiple vials to achieve the desired dose, repeat this step.



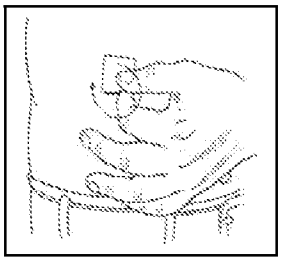
5. **Prepare the infusion pump and tubing** – Follow the manufacturer's instructions for preparing the pump, using subcutaneous administration sets and tubing, as needed. Be sure to prime the administration tubing to ensure that no air is left in the tubing.

6. **Select the injection site(s)** – The number and location of injection sites depends on the volume of the total dose. Infuse Hizentra into a maximum of 4 sites simultaneously; each injection site should be at least 2 inches apart.

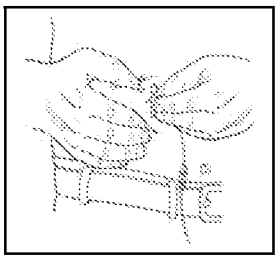


7. **Clean the injection site(s)** – Using an antiseptic skin preparation, clean each site beginning at the center and working outward in a circular motion. Allow each site to dry before proceeding.

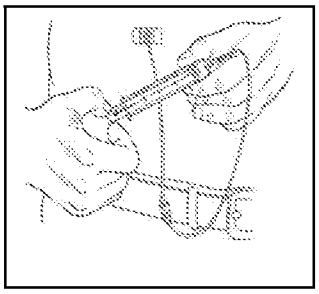
8. **Insert the needle** – Grasp the skin between 2 fingers and insert the needle into the subcutaneous tissue.



9. **Secure the needle to the skin** – If necessary, use sterile gauze and tape or transparent dressing to hold the needle in place.



10. **Check for proper placement of the needle** – Before starting the infusion, make sure no blood is flowing into the tubing. If blood is present, remove and discard the needle and administration tubing. Repeat the process from step 5 (priming) through step 10 (checking for proper needle placement) using a new needle, new administration tubing, and a different injection site.



11. **Infuse Hizentra** – Follow the manufacturer's instructions to turn on the pump.

12. **Record the infusion** – Remove the peel-off portion of the label from each vial used, and affix it to the patient's treatment diary/log book.

After administration, immediately discard any unused product and all used disposable supplies in accordance with local requirements.

For self-administration, provide the patient with instructions and training for subcutaneous infusion in the home or other appropriate setting.

3 DOSAGE FORMS AND STRENGTHS

Hizentra is a 0.2 g/mL (20%) protein solution for subcutaneous injection.

4 CONTRAINDICATIONS

Hizentra is contraindicated in patients who have had an anaphylactic or severe systemic reaction to the administration of human immune globulin or to components of Hizentra, such as polysorbate 80.

Hizentra is contraindicated in patients with hyperprolinemia because it contains the stabilizer L-proline (*see Description [11]*).

Hizentra is contraindicated in IgA-deficient patients with antibodies against IgA and a history of hypersensitivity (*see Description [11]*).

5 WARNINGS AND PRECAUTIONS

5.1 Hypersensitivity

Severe hypersensitivity reactions may occur to human immune globulin or components of Hizentra, such as polysorbate 80. In case of hypersensitivity, discontinue the Hizentra infusion immediately and institute appropriate treatment.

Individuals with IgA deficiency can develop anti-IgA antibodies and anaphylactic reactions (including anaphylaxis and shock) after administration of blood components containing IgA. Patients with known antibodies to IgA may have a greater risk of developing potentially severe hypersensitivity and anaphylactic reactions with administration of Hizentra. Hizentra contains ≤50 mcg/mL IgA (*see Description [11]*).

5.2 Reactions Reported to Occur With IGIV Treatment

The following reactions have been reported to occur with IGIV treatment and may occur with IGSC treatment.

Renal Dysfunction/Failure

Renal dysfunction/failure, osmotic nephropathy, and death may occur with use of human immune globulin products. Ensure that patients are not volume depleted and assess renal function, including measurement of blood urea nitrogen (BUN) and serum creatinine, before the initial infusion of Hizentra and at appropriate intervals thereafter.

Periodic monitoring of renal function and urine output is particularly important in patients judged to have a potential increased risk of developing acute renal failure.[1] If renal function deteriorates, consider discontinuing Hizentra. For patients judged to be at risk of developing renal dysfunction because of pre-existing renal insufficiency or predisposition to acute renal failure (such as those with diabetes mellitus or hypovolemia, those who are overweight or use concomitant nephrotoxic medicinal products, or those who are over 65 years of age), administer Hizentra at the minimum rate practicable.

Thrombotic Events

Thrombotic events may occur with use of human immune globulin products[2-4]. Patients at increased risk may include those with a history of atherosclerosis, multiple cardiovascular risk factors, advanced age, impaired cardiac output, hypercoagulable disorders, prolonged periods of immobilization, and/or known or suspected hyperviscosity. Because of the potentially increased risk of thrombosis, consider baseline assessment of blood viscosity in patients at risk for hyperviscosity, including those with cryoglobulins, fasting chylomicronemia/markedly high triacylglycerols (triglycerides), or monoclonal gammopathies. For patients judged to be at risk of developing thrombotic events, administer Hizentra at the minimum rate practicable.

Aseptic Meningitis Syndrome (AMS)

AMS may occur with use of human immune globulin products.[5] The syndrome usually begins within several hours to 2 days following IGIV treatment. AMS is characterized by signs and symptoms including severe headache, nuchal rigidity, drowsiness, fever, photophobia, painful eye movements, nausea, and vomiting. Cerebrospinal fluid (CSF) studies frequently show pleocytosis up to several thousand cells per cubic millimeter, predominantly from the granulocytic series, with elevated protein levels up to several hundred mg/dL. AMS may occur more frequently in association with high doses (2 g/kg) and/or rapid infusion of IGIV.

Conduct a thorough neurological examination, including CSF studies, to rule out other causes of meningitis in patients exhibiting signs and symptoms of AMS. Discontinuation of IGIV treatment has resulted in remission of AMS within several days without sequelae.

Hemolysis

Hizentra can contain blood group antibodies that may act as hemolysins and induce *in vivo* coating of red blood cells (RBCs) with immunoglobulin, causing a positive direct antiglobulin (Coombs') test result and hemolysis.[6-8] Delayed hemolytic anemia can develop subsequent to immune globulin therapy due to enhanced RBC sequestration, and acute hemolysis, consistent with intravascular hemolysis, has been reported.[9]

Monitor recipients of Hizentra for clinical signs and symptoms of hemolysis. If these are present after a Hizentra infusion, perform appropriate confirmatory laboratory testing. If transfusion is indicated for patients who develop hemolysis with clinically compromising anemia after receiving Hizentra, perform adequate cross-matching to avoid exacerbating on-going hemolysis.

Transfusion-Related Acute Lung Injury (TRALI)

Noncardiogenic pulmonary edema may occur in patients administered human immune globulin products.[10] TRALI is characterized by severe respiratory distress, pulmonary edema, hypoxemia, normal left ventricular function, and fever. Typically, it occurs within 1 to 6 hours following transfusion. Patients with TRALI may be managed using oxygen therapy with adequate ventilatory support.

Monitor Hizentra recipients for pulmonary adverse reactions. If TRALI is suspected, perform appropriate tests for the presence of anti-neutrophil antibodies in both the product and patient's serum.

5.3 Transmissible Infectious Agents

Because Hizentra is made from human plasma, it may carry a risk of transmitting infectious agents (e.g., viruses, and theoretically, the Creutzfeldt-Jakob disease [CJD] agent). The risk of infectious agent transmission has been reduced by screening plasma donors for prior exposure to certain viruses, testing for the presence of certain current virus infections, and including virus inactivation/removal steps in the manufacturing process for Hizentra.

Report all infections thought to be possibly transmitted by Hizentra to CSL Behring Pharmacovigilance at 1-866-915-6958.

5.4 Laboratory Tests

Various passively transferred antibodies in immunoglobulin preparations may lead to misinterpretation of the results of serological testing.

6 ADVERSE REACTIONS

The most common adverse reactions (ARs), observed in ≥5% of study subjects receiving Hizentra, were local reactions (i.e., swelling, redness, heat, pain, and itching at the injection site), headache, vomiting, pain, and fatigue.

6.1 Clinical Trials Experience

Because clinical studies are conducted under widely varying conditions, AR rates observed in clinical studies of a product cannot be directly compared to rates in the clinical studies of another product and may not reflect the rates observed in clinical practice.

The safety of Hizentra was evaluated in a clinical study for 15 months in subjects with PI who had been treated previously with IGIV every 3 or 4 weeks. The safety analyses included 49 subjects in the intention-to-treat (ITT) population. The ITT population consisted of all subjects who received at least one dose of Hizentra (*see Clinical Studies [14]*).

Subjects were treated with Hizentra at weekly doses ranging from 66 to 331 mg/kg body weight during the wash-in/wash-out period and from 72 to 379 mg/kg during the efficacy period. The 49 subjects received a total of 2264 weekly infusions of Hizentra.

No deaths or serious ARs occurred during the study. Two subjects withdrew from the study due to ARs. One subject experienced a severe injection-site reaction one day after the third weekly infusion, and the other subject experienced moderate myositis. Both reactions were judged to be "at least possibly related" to the administration of Hizentra.

Table 2 summarizes the most frequent adverse events (AEs) (experienced by at least 4 subjects), *irrespective of causality*. Included are all AEs and those considered temporally associated with the Hizentra infusion, i.e., occurring during or within 72 hours after the end of an infusion. Local reactions were the most frequent AEs observed, with injection-site reactions (i.e., swelling, redness, heat, pain, and itching at the site of injection) comprising 98% of local reactions.

Table 2: Incidence of Subjects With Adverse Events (AEs)* (Experienced by 4 or More Subjects) and Rate per Infusion, *Irrespective of Causality* (ITT Population)

AE (≥4 Subjects)	All AEs*		AEs* Occurring During or Within 72 Hours of Infusion	
	Number (%) of Subjects (n=49)	Number (Rate†) of AEs (n=2264 Infusions)	Number (%) of Subjects (n=49)	Number (Rate†) of AEs (n=2264 Infusions)
Local reactions‡	49 (100)	1340 (0.592)	49 (100)	1322 (0.584)
Other AEs:				
Headache	13 (26.5)	40 (0.018)	12 (24.5)	32 (0.014)
Cough	8 (16.3)	9 (0.004)	5 (10.2)	6 (0.003)
Diarrhea	7 (14.3)	8 (0.004)	5 (10.2)	6 (0.003)
Fatigue	6 (12.2)	6 (0.003)	4 (8.2)	4 (0.002)
Back pain	5 (10.2)	11 (0.005)	4 (8.2)	5 (0.002)
Nausea	5 (10.2)	5 (0.002)	4 (8.2)	4 (0.002)
Abdominal pain, upper	5 (10.2)	5 (0.002)	3 (6.1)	3 (0.001)
Rash	5 (10.2)	7 (0.003)	2 (4.1)	3 (0.001)
Pain in extremity	4 (8.2)	7 (0.003)	4 (8.2)	6 (0.003)
Migraine	4 (8.2)	5 (0.002)	3 (6.1)	4 (0.002)
Pain	4 (8.2)	5 (0.002)	3 (6.1)	4 (0.002)
Epistaxis	4 (8.2)	6 (0.003)	2 (4.1)	3 (0.001)
Pharyngolaryngeal pain	4 (8.2)	6 (0.003)	2 (4.1)	2 (<0.001)
Arthralgia	4 (8.2)	5 (0.002)	2 (4.1)	3 (0.001)

* Excluding infections.

† Rate of AEs per infusion.

‡ Includes injection-site reactions as well as bruising, scabbing, pain, irritation, cysts, eczema, and nodules at the injection site.

The ratio of infusions with temporally associated AEs, including local reactions, to all infusions was 1338 to 2264 (59.1%; upper 95% confidence limit of 62.4%). Excluding local reactions, the corresponding ratio was 173 to 2264 (7.6%; upper 95% confidence limit of 8.9%).

Table 3 summarizes the most frequent ARs (i.e., those AEs considered by the investigators to be "at least possibly related" to Hizentra administration) experienced by at least 2 subjects.

Table 3: Incidence of Subjects With Adverse Reactions (Experienced by 2 or More Subjects) to Hizentra and Rate per Infusion (ITT Population)

Adverse Reaction (≥2 Subjects)	Number (%) of Subjects (n=49)	Number (Rate*) of Adverse Reactions (n=2264 Infusions)
Local reactions†	49 (100)	1338 (0.591)
Other ARs:		
Headache	12 (24.5)	36 (0.016)
Vomiting	3 (6.1)	3 (0.001)
Pain	3 (6.1)	4 (0.002)
Fatigue	3 (6.1)	3 (0.001)
Contusion	2 (4.1)	3 (0.001)
Back pain	2 (4.1)	3 (0.001)
Migraine	2 (4.1)	3 (0.001)
Diarrhea	2 (4.1)	2 (<0.001)
Abdominal pain, upper	2 (4.1)	2 (<0.001)
Nausea	2 (4.1)	2 (<0.001)
Rash	2 (4.1)	2 (<0.001)
Arthralgia	2 (4.1)	2 (<0.001)

* Rate of ARs per infusion.
† Includes injection-site reactions as well as bruising, scabbing, pain, irritation, cysts, eczema, and nodules at the injection site.

Table 4 summarizes injection-site reactions based on investigator assessments 15 to 45 minutes after the end of the 683 infusions administered during regularly scheduled visits (every 4 weeks).

Table 4: Investigator Assessments* of Injection-Site Reactions by Infusion

Injection-Site Reaction	Number† (Rate‡) of Reactions (n=683 Infusions§)
Edema/induration	467 (0.68)
Erythema	346 (0.50)
Local heat	108 (0.16)
Local pain	88 (0.13)
Itching	64 (0.09)

* 15 to 45 minutes after the end of infusions administered at regularly scheduled visits (every 4 weeks).
† For multiple injection sites, every site was judged, but only the site with the strongest reaction was recorded.
‡ Rate of injection-site reactions per infusion.
§ Number of infusions administered during regularly scheduled visits.

Most local reactions were either mild (93.4%) or moderate (6.3%) in intensity.

6.2 Postmarketing Experience

Because postmarketing reporting of adverse reactions is voluntary and from a population of uncertain size, it is not always possible to reliably estimate the frequency of these reactions or establish a causal relationship to product exposure.

The following adverse reactions have been identified and reported during the postmarketing use of IGIV products[11]:

- *Infusion reactions:* Hypersensitivity (e.g., anaphylaxis), headache, diarrhea, tachycardia, fever, fatigue, dizziness, malaise, chills, flushing, urticaria or other skin reactions, wheezing or other chest discomfort, nausea, vomiting, rigors, back pain, myalgia, arthralgia, and changes in blood pressure
- *Renal:* Acute renal dysfunction/failure, osmotic nephropathy
- *Respiratory:* Apnea, Acute Respiratory Distress Syndrome (ARDS), TRALI, cyanosis, hypoxemia, pulmonary edema, dyspnea, bronchospasm
- *Cardiovascular:* Cardiac arrest, thromboembolism, vascular collapse, hypotension
- *Neurological:* Coma, loss of consciousness, seizures, tremor, aseptic meningitis syndrome
- *Integumentary:* Stevens-Johnson syndrome, epidermolysis, erythema multiforme, dermatitis (e.g., bullous dermatitis)
- *Hematologic:* Pancytopenia, leukopenia, hemolysis, positive direct antiglobulin (Coombs') test
- *Gastrointestinal:* Hepatic dysfunction, abdominal pain
- *General/Body as a Whole:* Pyrexia, rigors

To report SUSPECTED ADVERSE REACTIONS, contact CSL Behring Pharmacovigilance at 1-866-915-6958 or FDA at 1-800-FDA-1088 or *www.fda.gov/medwatch.*

7 DRUG INTERACTIONS

7.1 Live Virus Vaccines

The passive transfer of antibodies with immunoglobulin administration may interfere with the response to live virus vaccines such as measles, mumps, rubella, and varicella (*see Patient Counseling Information [17]*).

7.2 Serological Testing

Various passively transferred antibodies in immunoglobulin preparations may lead to misinterpretation of the results of serological testing.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy
Pregnancy Category C. Animal reproduction studies have not been conducted with Hizentra. It is not known whether Hizentra can cause fetal harm when administered to a pregnant woman or can affect reproduction capacity. Hizentra should be given to pregnant women only if clearly needed.

8.3 Nursing Mothers
Hizentra has not been evaluated in nursing mothers.

8.4 Pediatric Use
Hizentra was evaluated in 10 pediatric subjects (3 children and 7 adolescents) with PI. No pediatric-specific dose requirements were necessary to achieve the desired serum IgG levels.

Hizentra was not evaluated in neonates or infants.

8.5 Geriatric Use
Of the 49 subjects evaluated in the clinical study of Hizentra, 6 subjects were 65 years of age or older. No overall differences in safety or efficacy were observed between these subjects and younger subjects.

11 DESCRIPTION
Hizentra, Immune Globulin Subcutaneous (Human), 20% Liquid, is a ready-to-use, sterile 20% (0.2 g/mL) protein liquid preparation of polyvalent human immunoglobulin G (IgG) for subcutaneous administration. Hizentra is manufactured from large pools of human plasma by a combination of cold alcohol fractionation, octanoic acid fractionation, and anion exchange chromatography. The IgG proteins are not subjected to heating or to chemical or enzymatic modification. The Fc and Fab functions of the IgG molecule are retained. Fab functions tested include antigen binding capacities, and Fc functions tested include complement activation and Fc-receptor-mediated leukocyte activation (determined with complexed IgG).

Hizentra has a purity of ≥98% IgG and a pH of 4.6 to 5.2. Hizentra contains approximately 250 (range: 210 to 290 mmol/L) L-proline (a nonessential amino acid) as a stabilizer, 10 to 30 mg/L polysorbate 80, and trace amounts of sodium. Hizentra contains ≤50 mcg/mL IgA. Hizentra contains no carbohydrate stabilizers (e.g., sucrose, maltose) and no preservative.

Plasma units used in the manufacture of Hizentra are tested using FDA-licensed serological assays for hepatitis B surface antigen and antibodies to human immunodeficiency virus (HIV)-1/2 and hepatitis C virus (HCV) as well as FDA-licensed Nucleic Acid Testing (NAT) for HIV-1 and HCV. All plasma units have been found to be nonreactive (negative) in these tests. For hepatitis B virus (HBV), an investigational NAT procedure is used and the plasma units found to be negative; however, the significance of a negative result has not been established. In addition, the plasma has been tested for B19 virus (B19V) DNA by NAT. Only plasma that passes virus

screening is used for production, and the limit for B19V in the fractionation pool is set not to exceed 10^4 IU of B19V DNA per mL.

The manufacturing process for Hizentra includes three steps to reduce the risk of virus transmission. Two of these are dedicated virus clearance steps: pH 4 incubation to inactivate enveloped viruses and virus filtration to remove, by size exclusion, both enveloped and non-enveloped viruses as small as approximately 20 nanometers. In addition, a depth filtration step contributes to the virus reduction capacity.[12]

These steps have been independently validated in a series of *in vitro* experiments for their capacity to inactivate and/or remove both enveloped and non-enveloped viruses. Table 5 shows the virus clearance during the manufacturing process for Hizentra, expressed as the mean $\log_{10}$ reduction factor (LRF).

Table 5: Virus Inactivation/Removal in Hizentra*

	HIV-1	PRV	BVDV	WNV	EMCV	MVM
Virus Property						
Genome	RNA	DNA	RNA	RNA	RNA	DNA
Envelope	Yes	Yes	Yes	Yes	No	No
Size (nm)	80-100	120-200	50-70	50-70	25-30	18-24
Manufacturing Step	**Mean LRF**					
pH 4 incubation	≥5.4	≥5.9	4.6	≥7.8	nt	nt
Depth filtration	≥5.3	≥6.3	2.1	3.0	4.2	2.3
Virus filtration	≥5.3	≥5.5	≥5.1	≥5.9	≥5.4	≥5.5
Overall Reduction ($\log_{10}$ Units)	**≥16.0**	**≥17.7**	**≥11.8**	**≥16.7**	**≥9.6**	**≥7.8**

HIV-1, human immunodeficiency virus type 1, a model for HIV-1 and HIV-2; PRV, pseudorabies virus, a nonspecific model for large enveloped DNA viruses (e.g., herpes virus); BVDV, bovine viral diarrhea virus, a model for hepatitis C virus; WNV, West Nile virus; EMCV, encephalomyocarditis virus, a model for hepatitis A virus; MVM, minute virus of mice, a model for a small highly resistant non-enveloped DNA virus (e.g., parvovirus); LRF, $\log_{10}$ reduction factor; nt, not tested; na, not applicable.
* The virus clearance of human parvovirus B19 was investigated experimentally at the pH 4 incubation step. The estimated LRF obtained was ≥5.3.

The manufacturing process was also investigated for its capacity to decrease the infectivity of an experimental agent of transmissible spongiform encephalopathy (TSE), considered a model for CJD and its variant (vCJD).[12] Several of the production steps have been shown to decrease infectivity of an experimental TSE model agent. TSE reduction steps include octanoic acid fractionation (≥6.4 $\log_{10}$), depth filtration (2.6 $\log_{10}$), and virus filtration (≥5.8 $\log_{10}$). These studies provide reasonable assurance that low levels of vCJD/CJD agent infectivity, if present in the starting material, would be removed.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Hizentra supplies a broad spectrum of opsonizing and neutralizing IgG antibodies against a wide variety of bacterial and viral agents. The mechanism of action in PI has not been fully elucidated.

12.3 Pharmacokinetics

The pharmacokinetics (PK) of Hizentra was evaluated in a PK substudy of subjects with PI participating in the 15-month efficacy and safety study (*see Clinical Studies [14]*). All PK subjects were treated previously with Privigen®, Immune Globulin Intravenous (Human), 10% Liquid and were switched to weekly subcutaneous treatment with Hizentra. After a 3-month wash-in/wash-out period, doses were adjusted individually with the goal of providing a systemic serum IgG exposure (area under the IgG serum concentration vs time curve; AUC) not inferior to that of the previous weekly-equivalent IGIV dose. Table 6 summarizes PK parameters for subjects in the substudy following treatment with Hizentra and IGIV.

Table 6: Pharmacokinetics Parameters of Hizentra and IGIV

	Hizentra	**IGIV* (Privigen®)**
Number of subjects	18	18
Dose*		
Mean	228 mg/kg bw	152 mg/kg bw
Range	141-381 mg/kg bw	86-254 mg/kg bw
IgG peak levels		
Mean	1616 mg/dL	2564 mg/dL
Range	1090-2825 mg/dL	2046-3456 mg/dL
IgG trough levels		
Mean	1448 mg/dL	1127 mg/dL
Range	952-2623 mg/dL	702-1810 mg/dL
AUC†		
Mean	10560 day x mg/dL	10320 day x mg/dL
Range	7210-18670 day x mg/dL	8051-15530 day x mg/dL

bw, body weight.
* For IGIV: weekly-equivalent dose.
† Standardized to a 7-day period.

For the 19 subjects completing the wash-in/wash-out period, the average dose adjustment for Hizentra was 153% (range: 126% to 187%) of the previous weekly-equivalent IGIV dose. After 12 weeks of treatment with Hizentra at this individually adjusted dose, the final steady-state AUC determinations were made in 18 of the 19 subjects. The geometric mean ratio of the steady-state AUCs, standardized to a weekly treatment period, for Hizentra vs IGIV treatment was 1.002 (range: 0.77 to 1.20) with a 90% confidence limit of 0.951 to 1.055 for the 18 subjects.

The PK study included an additional assessment to determine the ratio of serum IgG trough levels with Hizentra (IGSC) compared to the previous trough levels with IGIV that were associated with matching AUCs. It was demonstrated that IgG trough levels during treatment

with Hizentra were 1.3 times higher than the preceding trough levels during treatment with IGIV (Privigen®). This calculated IGSC:IGIV ratio of 1.3 (±15% of this value, or ±0.2) can be used to assess dosing with Hizentra by providing a steady-state target IgG trough level, which may be assumed to be within the range of 1.1 to 1.5 times the previous steady-state trough levels with IGIV. However, the patient's clinical response should be the primary consideration in dose adjustment (*see Dosage and Administration [2.2]*).

With Hizentra, peak serum levels are lower (1616 vs 2564 mg/dL) than those achieved with IGIV while trough levels are generally higher (1448 vs 1127 mg/dL). In contrast to IGIV administered every 3 to 4 weeks, weekly subcutaneous administration results in relatively stable steady-state serum IgG levels.[13,14] After the subjects had reached steady-state with weekly administration of Hizentra, peak serum IgG levels were observed after a mean of 2.9 days (range: 0 to 7 days) in 18 subjects.

13 NONCLINICAL TOXICOLOGY

13.2 Animal Toxicology and/or Pharmacology

Long- and short-term memory loss was seen in juvenile rats in a study modeling hyperprolinemia. In this study, rats received daily subcutaneous injections with L-proline from day 6 to day 28 of life.[15] The daily amounts of L-proline used in this study were more than 60 times higher than the L-proline dose that would result from the administration of 400 mg/kg body weight of Hizentra once weekly. In unpublished studies using the same animal model (i.e., rats) dosed with the same amount of L-proline with a dosing interval relevant to IGSC treatment (i.e., on 5 consecutive days on days 9 to 13, or once weekly on days 9, 16, and 23), no effects on learning and memory were observed. The clinical relevance of these studies is not known.

14 CLINICAL STUDIES

A prospective, open-label, multicenter, single-arm, clinical study conducted in the US evaluated the efficacy, tolerability, and safety of Hizentra in adult and pediatric subjects with PI. Subjects previously receiving monthly treatment with IGIV were switched to weekly subcutaneous administration of Hizentra for 15 months (a 3-month wash-in/wash-out period followed by a 12-month efficacy period). The efficacy analyses included 38 subjects in the modified intention-to-treat (MITT) population. The MITT population consisted of subjects who completed the wash-in/wash-out period and received at least one infusion of Hizentra during the efficacy period.

Although 5% of the administered doses could not be verified, the weekly doses of Hizentra ranged from 72 to 379 mg per kg body weight, which was 149% (range: 114% to 180%) of the previous IGIV dose. Subjects received a total of 2264 infusions of Hizentra.

In the study, the number of injection sites per infusion ranged from 1 to 12. In 73% of infusions; the number of injection sites was 4 or fewer. Up to 4 simultaneous injection sites were permitted using 2 pumps; however, more than 4 sites could be used consecutively during one infusion. The infusion flow rate did not exceed 50 mL per hour for all injection sites combined. During the efficacy period, the median duration of a weekly infusion ranged from 1.6 to 2.0 hours.

The study evaluated the annual rate of serious bacterial infections (SBIs), defined as bacterial pneumonia, bacteremia/septicemia, osteomyelitis/septic arthritis, bacterial meningitis, and visceral abscess. The study also evaluated the annual rate of any infections, the use of antibiotics for infection (prophylaxis or treatment), the days out of work/school/kindergarten/day care or unable to perform normal activities due to infections, and hospitalizations due to infections.

Table 7 summarizes the efficacy results for subjects in the efficacy phase (MITT population) of the study. No subjects experienced an SBI in this study.

Table 7: Summary of Efficacy Results (MITT Population)

Number of subjects (efficacy phase)	38
Total number of subject days	12,697
Infections Annual rate of SBIs* Annual rate of any infections	 0 SBIs per subject year† 2.76 infections/subject year‡
Antibiotic use for infection (prophylaxis or treatment) Number of subjects (%) Annual rate	 27 (71.1) 48.5 days/subject year
Total number of subject days	12,605
Days out of work/school/kindergarten/day care or unable to perform normal activities due to infections Number of days (%) Annual rate	 71 (0.56) 2.06 days/subject year
Hospitalizations due to infections Number of days (%) Annual rate	 7 (0.06)§ 0.2 days/subject year

* Defined as bacterial pneumonia, bacteremia/septicemia, osteomyelitis/septic arthritis, bacterial meningitis, and visceral abscess.
† Upper 99% confidence limit: 0.132.
‡ 95% confidence limits: 2.235; 3.370.
§ Based on 1 subject.

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16 HOW SUPPLIED/STORAGE AND HANDLING

16.1 How Supplied

Hizentra is supplied in a single-use, tamper-evident vial containing 0.2 grams of protein per mL of preservative-free liquid. Each vial label contains a peel-off strip with the vial size and product lot number for use in recording doses in a patient treatment record.

The components used in the packaging for Hizentra contain no latex.

The following dosage presentations are available:

NDC Number	Fill Size (mL)	Grams Protein
44206-451-01	5 mL	1
44206-452-02	10 mL	2
44206-454-04	20 mL	4

16.2 Storage and Handling

When stored at room temperature (up to 25°C [77°F]), Hizentra is stable for the period indicated by the expiration date printed on the outer carton and vial label. DO NOT FREEZE. Do not use product that has been frozen. Do not shake. Keep Hizentra in its original carton to protect it from light.

17 PATIENT COUNSELING INFORMATION

- **Self-administration** – If self-administration is appropriate, ensure that the patient receives instructions and training on subcutaneous administration in the home or other appropriate setting and has demonstrated the ability to perform subcutaneous infusions.
  - Ensure patients understand the importance of adhering to the weekly administration schedule to maintain the steady levels of IgG in their blood.
  - Instruct patients to keep their treatment diary/log book current by recording, after each infusion, the time, date, dose, and any reactions, and by removing the peel-off portion of the label (containing the lot number) from the product vial and placing it in the treatment diary/log book.
  - Tell patients that mild to moderate local (injection-site) reactions (e.g., swelling and redness) are a common side effect of subcutaneous therapy, but to contact their healthcare professional if a local reaction persists for more than a few days.
  - Inform patients of the importance of having an infusion needle long enough to reach the subcutaneous tissue and of changing the actual site of injection with each infusion.
  - Inform patients to consider adjusting the injection-site location, volume per site, and rate of infusion based on how infusions are tolerated.

- **Dose adjustments** – Inform patients that they should be tested regularly to make sure they have the correct levels of Hizentra (IgG) in their blood. These tests may result in adjustments to the Hizentra dose.

- **Hypersensitivity** – Inform patients of the early signs of hypersensitivity reactions to Hizentra (including hives, generalized urticaria, tightness of the chest, wheezing, hypotension, and anaphylaxis), and advise them to notify their physician if they experience any of these symptoms.

- **Interference With Vaccines** – Inform patients that administration of IgG may interfere with the response to live virus vaccines (e.g., measles, mumps, rubella, and varicella) and to notify their immunizing physician of recent therapy with Hizentra.

- **Reactions reported to occur with IGIV treatment** – Advise patients to be aware of and immediately report to their physician symptoms of the following potential reactions:
  - Decreased urine output, sudden weight gain, fluid retention/edema, and/or shortness of breath, which may suggest kidney problems
  - Shortness of breath, changes in mental status, chest pain, and other manifestations of thrombotic and embolic events
  - Severe headache, neck stiffness, drowsiness, fever, sensitivity to light, painful eye movements, nausea, and vomiting, which may suggest aseptic meningitis syndrome
  - Fatigue, increased heart rate, yellowing of the skin or eyes, and dark-colored urine, which may suggest hemolysis
  - Severe breathing problems, lightheadedness, drops in blood pressure, and fever, which may suggest TRALI (a condition typically occurring within 1 to 6 hours following transfusion)

- **Transmissible infectious agents** – Inform patients that Hizentra is made from human plasma (part of the blood) and may contain infectious agents that can cause disease (e.g., viruses and, theoretically, the CJD agent). Explain that the risk that Hizentra may transmit an infectious agent has been reduced by screening the plasma donors, by testing the donated plasma for certain virus infections, and by inactivating and/or removing certain viruses during manufacturing.

The attached Hizentra "Information for Patients" contains more detailed instructions for patients who will be self-administering Hizentra.

Hizentra
Immune Globulin Subcutaneous (Human), 20% Liquid

Information for Patients
This patient package insert summarizes important information about Hizentra. Please read it carefully before using this medicine. This information does not take the place of talking with your healthcare professional, and it does not include all of the important information about Hizentra. If you have any questions after reading this, ask your healthcare professional.

What is the most important information I should know about Hizentra?
Hizentra is supposed to be infused under your skin only. DO NOT inject Hizentra into a blood vessel (vein or artery).

What is Hizentra?
Hizentra (Hi – ZEN – tra) is a prescription medicine used to treat primary immune deficiency (PI). Hizentra is made from human plasma. It contains antibodies, called immunoglobulin G (IgG), that healthy people have to fight germs (bacteria and viruses).

People with PI get a lot of infections. Hizentra helps lower the number of infections you will get.

Who should NOT take Hizentra?
Do not take Hizentra if you have too much proline in your blood (called "hyperprolinemia") or if you have had reactions to polysorbate 80.

Tell your doctor if you have had a serious reaction to other immune globulin medicines or if you have been told that you also have a deficiency of the immunoglobulin called IgA.

How should I take Hizentra?
You will take Hizentra through an infusion under your skin. You will use up to 4 needles that are put into different places of your body at one time. The needles are attached to a pump with an infusion tube. It usually takes about 60 minutes to do one infusion. You will need to have infusions once a week.

Instructions for using Hizentra are at the end of this patient package insert (see "How do I use Hizentra?"). Do not use Hizentra by yourself until you have been taught how by your doctor or healthcare professional.

What should I avoid while taking Hizentra?
Vaccines may not work well for you while you are taking Hizentra. Tell your doctor or healthcare professional that you are taking Hizentra before you get a vaccine.

Tell your doctor or healthcare professional if you are pregnant or plan to become pregnant, or if you are nursing.

What are possible side effects of Hizentra?
The most common side effects with Hizentra are:

- Redness, swelling, and itching at the injection site
- Headache/migraine
- Vomiting
- Pain (including pain in the back, joints, arms, legs)
- Fatigue
- Bruising
- Diarrhea
- Stomach ache
- Nausea
- Rash

Tell your doctor right away or go to the emergency room if you have hives, trouble breathing, wheezing, dizziness, or fainting. These could be signs of a bad allergic reaction.

Tell your doctor right away if you have any of the following symptoms. They could be signs of a serious problem.

- Reduced urination, sudden weight gain, or swelling in your legs. These could be signs of a kidney problem.
- Pain, swelling, warmth, redness, or a lump in your legs or arms. These could be signs of a blood clot.
- Bad headache with nausea, vomiting, stiff neck, fever, and sensitivity to light. These could be signs of a brain swelling called meningitis.
- Brown or red urine, fast heart rate, yellow skin or eyes. These could be signs of a blood problem.
- Chest pains or trouble breathing.
- Fever over 100°F. This could be a sign of an infection.

Tell your doctor about any side effects that concern you. You can ask your doctor to give you more information that is available to healthcare professionals.

How do I use Hizentra?
Infuse Hizentra only after you have been trained by your doctor or healthcare professional. Below are step-by-step instructions to help you remember how to use Hizentra. Ask your doctor or healthcare professional about any instructions you do not understand.

Instructions for use
Hizentra comes in single-use vials.

Keep Hizentra in the storage box at room temperature.

Step 1: Gather the Hizentra vials, the following disposable supplies (not provided with Hizentra), and other items (infusion pump, sharps container, treatment diary or logbook):

- Infusion administration tubing
- Needle or catheter sets (for subcutaneous infusion)
- Y-site connectors (if needed)
- Alcohol wipes
- Antiseptic skin preps
- Syringes
- Transfer needles
- Gauze and tape, or transparent dressing
- Gloves (if recommended by your doctor)

Step 2: Wash hands

- Thoroughly wash and dry your hands (Figure 1).
- If you have been told to wear gloves when preparing your infusion, put the gloves on.

Figure 1

Step 3: Clean surface

Thoroughly clean a table or other flat surface using one of the alcohol wipes.

Step 4: Check vials

Carefully look at the liquid in each vial of Hizentra (Figure 2). It should look clear and be pale yellow to light brown. Check for particles or color changes. **Do not use the vial if**:

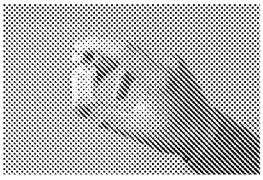

Figure 2

- The liquid looks cloudy, contains particles, or has changed color.
- The protective cap is missing.
- The expiration date on the label has passed.

Step 5: Transfer Hizentra to syringe for infusion

- Take the protective cap off the vial (Figure 3).

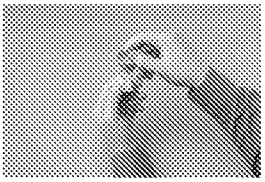

Figure 3

- Clean the vial stopper with an alcohol wipe (Figure 4). Let the stopper dry.

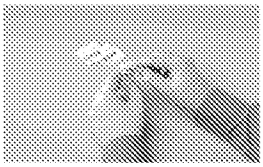

Figure 4

- Attach a transfer needle to the syringe tip (Figure 5).

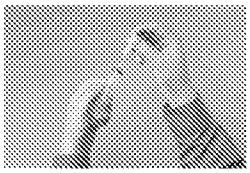
Figure 5

- Pull out the plunger of the syringe to fill the syringe with air. The amount of air should be the same as the amount of Hizentra you will transfer from the vial.
- Put the Hizentra vial on a flat surface. Keeping the vial upright, insert the transfer needle into the center of the rubber stopper.
- Check that the tip of the needle is not in the liquid. Then, push the plunger of the syringe down. This will inject the air from the syringe into the airspace of the vial.
- Leaving the needle in the stopper, carefully turn the vial upside down (Figure 6).
- Slowly pull back on the plunger of the syringe to fill the syringe with Hizentra.
- Take the filled syringe and needle out of the stopper. Take off the needle and throw it away in the sharps container.

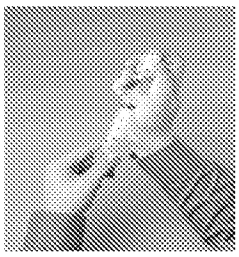
Figure 6

Step 6: Prepare pump and infusion tubing

Prepare the infusion pump (following the manufacturer's instructions) and prime (fill) the infusion tubing. To prime the tubing, connect the syringe filled with Hizentra to the infusion tubing and gently push on the syringe plunger to fill the tubing with Hizentra (Figure 7).

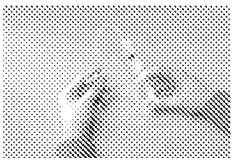
Figure 7

Step 7: Prepare injection site(s)

- Select an area on your abdomen, thigh, upper arm, or side of upper leg/hip for the infusion (Figure 8).
- Use a different site from the last time you infused Hizentra. New sites should be at least 1 inch from a previous site.
- Never infuse into areas where the skin is tender, bruised, red, or hard. Avoid infusing into scars or stretch marks.
- If you are using more than one injection site, be sure each site is at least 2 inches apart.
- During an infusion, do not use more than 4 injection sites at the same time.

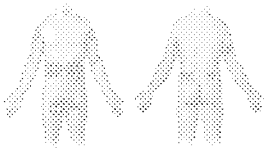
Figure 8

- Clean the skin at each site with an antiseptic skin prep (Figure 9). Let the skin dry.

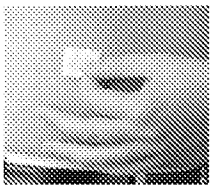
Figure 9

Step 8: Insert needle(s)

- With two fingers, pinch together the skin around the injection site. Insert the needle under the skin (Figure 10).

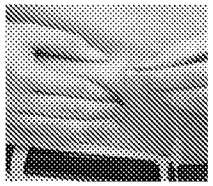
Figure 10

- Put sterile gauze and tape or a transparent dressing over the injection site (Figure 11). This will keep the needle from coming out.

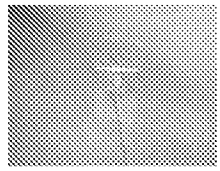
Figure 11

- Make sure you are not injecting Hizentra into a blood vessel. To test for this, attach a sterile syringe to the end of the infusion tubing. Pull the plunger back gently (Figure 12). If you see any blood in the tubing, take the needle out of the injection site. Throw away the tubing and needle. Start the infusion over at a different site with new infusion tubing and a new needle.

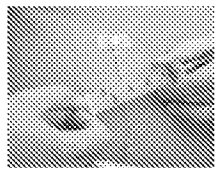
Figure 12

Step 9: Start infusion

Follow the instructions on the infusion pump (Figure 13) to connect the infusion tubing and set the infusion rate. (Your doctor will tell you what rate to use for your Hizentra infusion.) Turn on the pump.

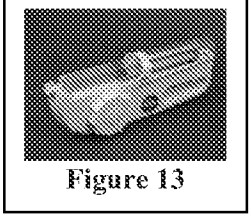
Figure 13

Step 10: Record treatment (Figure 14)

Peel off the removable part of the label of the Hizentra vial. Put this label in your treatment diary or logbook with the date and time of the infusion. Also include the exact amount of Hizentra that you infused.

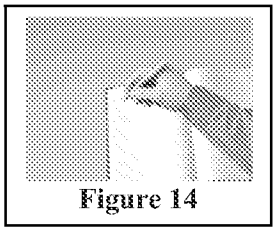
Figure 14

Step 11: Clean up

- When all the Hizentra has been infused, turn off the pump.
- Take off the dressing and take the needle out of the injection site.
- Throw away any Hizentra that is leftover in the single-use vial, along with the used disposable supplies, in the sharps container (Figure 15).
- Clean and store the infusion pump, following the manufacturer's instructions.

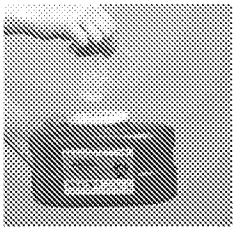

Figure 15

Be sure to tell your doctor about any problems you have doing your infusions. Your doctor may ask to see your treatment diary or logbook, so be sure to take it with you each time you visit the doctor's office.

Call your doctor for medical advice about side effects. You can also report side effects to FDA at 1-800-FDA-1088 or *www.fda.gov/medwatch.*

Manufactured by:
CSL Behring AG
Bern, Switzerland
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CSL Behring

Biotherapies for Life™

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CSL Behring Receives FDA Approval to Extend Shelf Life of Hizentra® from 18 Months to 24 Months

Added convenience for primary immunodeficiency patients using subcutaneous immunoglobulin at home

King of Prussia, PA — 18 August 2010

CSL Behring announced today that the U.S. Food and Drug Administration (FDA) has approved a supplemental Biologics License Application (sBLA) to extend the shelf life of Hizentra®, Immune Globulin Subcutaneous (Human), 20% Liquid, from 18 months to 24 months. Hizentra, the first and only 20 percent subcutaneous immunoglobulin (SCIg) approved in the U.S. by the FDA, is also the first and only SCIg in the U.S. that may be stored at room temperature.

Hizentra is indicated for the treatment of primary immunodeficiency (PI). PI is a group of disorders, usually genetic, that result from a dysfunctional immune system. This condition prevents patients from fighting off infections caused by common germs.

Stabilized with L-proline, a naturally occurring amino acid, **Hizentra** can be stored at room temperature (up to 25°C [77°F]) for up to 24 months. Because no refrigeration is necessary, **Hizentra** is always ready to use without warming, offering patients and physicians convenience and portability.

"CSL Behring continually enhances the products in our broad portfolio based on what we know patients and their healthcare providers need and want," said Robert Lefebvre, Vice President and General Manager, U.S. Commercial Operations at CSL Behring. "The ability to store **Hizentra** without refrigeration for up to two years is an innovation that can positively impact busy and active patients managing their primary immunodeficiency at home or in any setting that is convenient for them."

The sBLA for **Hizentra** was based on a study assessing the product's stability. Physicochemical, biological and immunological parameters were assessed over 24 months' storage under controlled conditions at 25°C (77°F). The data generated from this study support that when **Hizentra** is stored at room temperature (up to 25°C [77°F]) and protected from light, it is stable for up to 24 months.

Hizentra with 24-month shelf life packaging is expected to be available later this year. Current **Hizentra** patients are encouraged to contact their physician with any questions regarding the shelf life of their current supply.

Hizentra is part of the immunoglobulin (Ig) franchise for CSL Behring. This comprehensive Ig product portfolio also includes the first U.S. FDA-approved subcutaneous immunoglobulin and the first proline-stabilized intravenous immunoglobulin. CSL Behring manufactures **Hizentra** at its state-of-the art facility in Bern, Switzerland, where advanced technologies are applied to further ensure product safety and ample supply. This facility represents the long-term commitment of CSL Behring to global Ig markets.

For more information about **Hizentra**, visit www.hizentra.com.

Important Safety Information

Hizentra®, Immune Globulin Subcutaneous (Human) is indicated for the treatment of patients with primary immunodeficiency (PI).

Hizentra is contraindicated in individuals with a history of anaphylactic or severe systemic response to immune globulin preparations or components of **Hizentra**, and in persons with selective immunoglobulin A deficiency who have known antibody against IgA and a history of hypersensitivity. If anaphylactic reactions are suspected, administration should be discontinued immediately and the patient treated as medically appropriate. Because **Hizentra** contains the stabilizer L-proline, it is also contraindicated in patients with hyperprolinemia.

Hizentra is derived from human plasma. The risk of transmission of infectious agents including viruses and, theoretically, the Creutzfeldt-Jakob disease (CJD) agent, cannot be eliminated completely.

The most common drug-related adverse reactions, observed in 5 percent or more of subjects in the clinical study, were local injection-site reactions, headache, vomiting, pain, and fatigue.

Monitor patients for reactions associated with IVIg treatment that might occur with **Hizentra**, including renal dysfunction/failure, thrombotic events, aseptic meningitis syndrome (AMS), hemolysis and transfusion-related acute lung injury (TRALI).

For more information, including full prescribing information, visit http://www.hizentra.com/prescribing-information.aspx.

About Primary Immunodeficiencies

Nearly 150 types of PIs exist.[1] For individuals with PI, many of them children, infections may not improve as expected with usual

treatments and may keep returning. As a result, patients may face repeated rounds of antibiotics or hospitalization for treatment. Repeated infections can lead to organ damage, which over time can become life-threatening.[2]

Collectively, PIs affect an estimated 10 million people worldwide[3], and the incidence is estimated to be 1 in 10,000[4]. Due to the X-linked inheritance in many PI syndromes, more males are affected than females[5]. For more information on PI, please visit www.cslbehring.com or contact the leading PI patient advocate groups in the U.S., the Immune Deficiency Foundation and the Jeffrey Modell Foundation.

About CSL Behring

CSL Behring is a leader in the plasma protein therapeutics industry. Committed to saving lives and improving the quality of life for people with rare and serious diseases, the company manufactures and markets a range of plasma-derived and recombinant therapies worldwide. CSL Behring therapies are indicated for the treatment of coagulation disorders including hemophilia and von Willebrand disease, primary immune deficiencies and inherited respiratory disease. The company's products are also used in cardiac surgery, organ transplantation, burn treatment and to prevent hemolytic diseases in newborns. CSL Behring operates one of the world's largest plasma collection networks, CSL Plasma. CSL Behring is a subsidiary of CSL Limited (ASX:CSL), a biopharmaceutical company headquartered in Melbourne, Australia. For more information, visit www.cslbehring.com.

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[4]Dube D; The challenge of immunodeficiency disorders. Postgraduate Medicine 2002.

[5]Patient UK. Immunodeficiency (Primary and Secondary). Available on http://www.patient.co.uk/doctor/Immunodeficiency-(Primary-and-Secondary).htm. Accessed August 2010.

PATENT
Customer No. 22,852
Attorney Docket No. 06478.1507-00000

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	
Reinhard **BOLLI** *et al.*	Group Art Unit: 1644
Application No.: 10/579,357	Examiner: Kim YUNSOO
Filed: May 16, 2006	
For: IMMUNOGLOBULIN PREPARATIONS HAVING INCREASED STABILITY	Confirmation No.: 2138

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

DECLARATION UNDER 37 C.F.R. § 1.132

I, Reinhard Bolli, do hereby make the following declaration:

1. I am a Swiss citizen, residing at Bellevuestr. 14, CH-3073 Gümligen, Switzerland.

2. I have been awarded a degree in Chemistry from the University of Basel and a Doctoral degree (Ph.D.) in phil. Nat. (Biochemistry) from the University of Bern (Switzerland).

3. I have been employed by CSL Behring since 01.10.1988 and am presently a senior manager of the Biochemistry department R&D at CSL Behring. During my employment at CSL Behring, I have been engaged in research and development regarding the investigation of plasma proteins including immunoglobulins.

4. I am an inventor of the subject matter in Application No. 10/579,357

5. I have read and understand Application No. 10/579,357, including the claims as amended in the response filed herewith. For instance, I understand that independent claim 1, as amended, now recites a stable immunoglobulin preparation, wherein the preparation comprises proline and wherein the preparation has a pH of 4.2 to 5.4 and wherein the preparation does not comprise nicotinamide. I also understand that independent claim 8, as amended, recites a stable immunoglobulin preparation, wherein the preparation comprises proline and has a pH of 4.2 to 5.4, and wherein the final concentration of proline is between 0.2 to 0.4 M.

6. I have read and understand the specification and claims of U.S. Patent No. 5,871,736 ("the '736 patent") directed to a immunoglobulin preparation.

7. The '736 patent teaches that "preferred stabilizers are compositions comprising nicotinamide together with one or more of the . . . amino acids or their derivatives." See the '736 patent, col. 4, lines 28-30. In addition, as shown in table 2 and table 5 of the '736 patent, proline was used in conjunction with nicotinamide and only in concentrations of up to 0.2 M. Proline was never disclosed, taught or suggested as sufficient to stabilize the composition in the absence of nicotinamide.

8. However, contrary to the teachings of the '736 patent, I have discovered, unexpectedly, that the use of proline alone and without nicotinamide is beneficial for immunoglobulin preparations. I also found, unexpectedly and apart from the teaching of the '736 patent, that proline at a final concentration between 0.2 to 0.4 M led to a decreased level of aggregate formation and coloring of immuoglobuline preparations.

9. In order to demonstrate the difference between the immunoglobulin preparations disclosed in the '736 patent and the present application, I prepared and

tested several immunoglobulin solutions, described below and shown in Tables 1-2 and Figures 1-3.

Comparative testing of IgG solutions with or without the addition of nicotinamide

10. CSL Behring purified IgG from pooled human plasma by cold ethanol and octanoic acid fractionation followed by anion exchange chromatography and concentrated IgG to approximately 100 mg/ml (10%) by ultrafiltration using large scale state of the art procedures. In my laboratory the 10% IgG solutions were formulated with or without L-proline and/or nicotinamide at concentrations of 0, 125, 250, 350 and 500 mmol/L at a pH of 4.8 ± 0.2 (See Table 1 below). These different formulations were then incubated at 40°C in the dark for up to 30 days. At day 0 and day 30 of the incubation, in my laboratory size exclusion HPLC with a TSK 3000SW column was used to analyze the percentage of aggregates in the different IgG formulations and UV/VIS photometry to measure the yellowish colouring (i.e. absorbance at 350nm) of the solutions. The results are shown in Table 1, Figure 1, and Figure 2.

Table 1: IgG-solutions (100mg/ml, pH 4.8 ± 0.2) were incubated at 40°C and analysed after 30 days storage.

Formulation		Aggregates (%)		Absorbance at 350nm	
Proline (mmol/L)	Nicotinamide (mmol/L)	Day 0	30d	day 0	30d
0	0	< 0.1	1.3	0.052	0.072
125	0	< 0.1	1.1	0.065	0.069
0	125	< 0.1	3.9	0.073	0.094
250	0	< 0.1	0.9	0.061	0.070
0	250	< 0.1	10.3	0.084	0.116
350	0	< 0.1	0.7	0.069	0.066
0	350	< 0.1	19.5	0.087	0.157
500	0	< 0.1	0.6	0.086	0.064
0	500	0.4	37.2	0.082	0.271
125	125	< 0.1	3.0	0.075	0.090
250	250	< 0.1	7.7	0.085	0.105
350	350	< 0.1	13.7	0.097	0.129
500	500	0.2	26.1	0.102	0.186

Figure 1: Aggregate formation of 10% IgG solutions after storage at 40°C for 30 days.

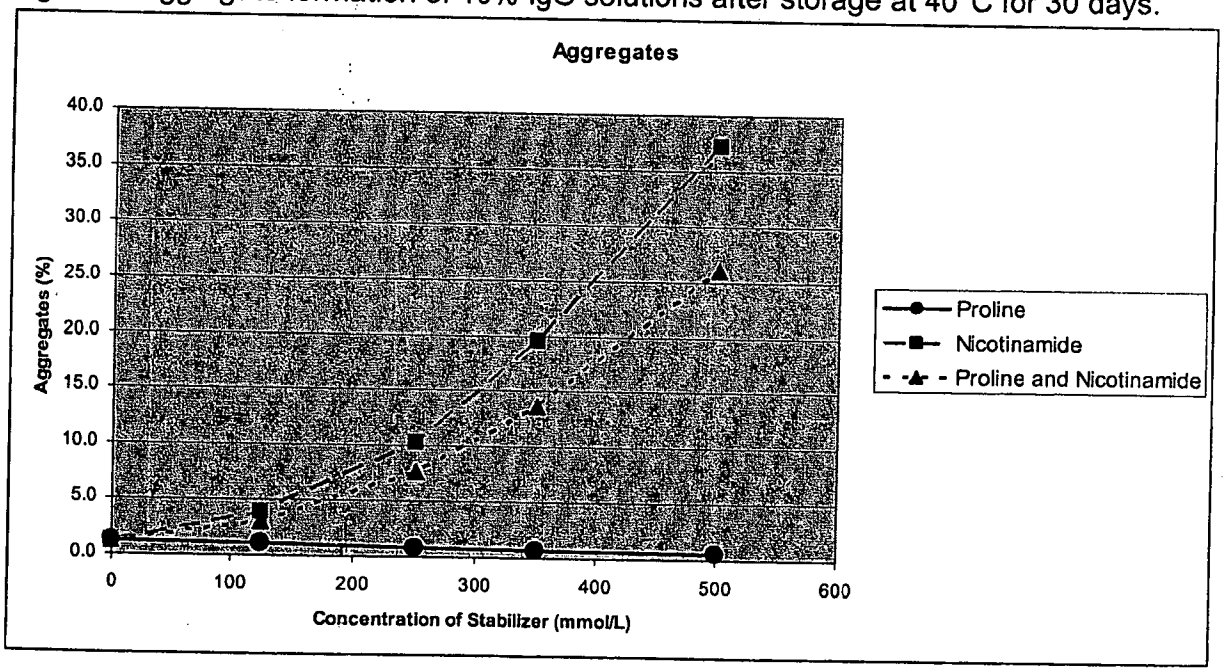
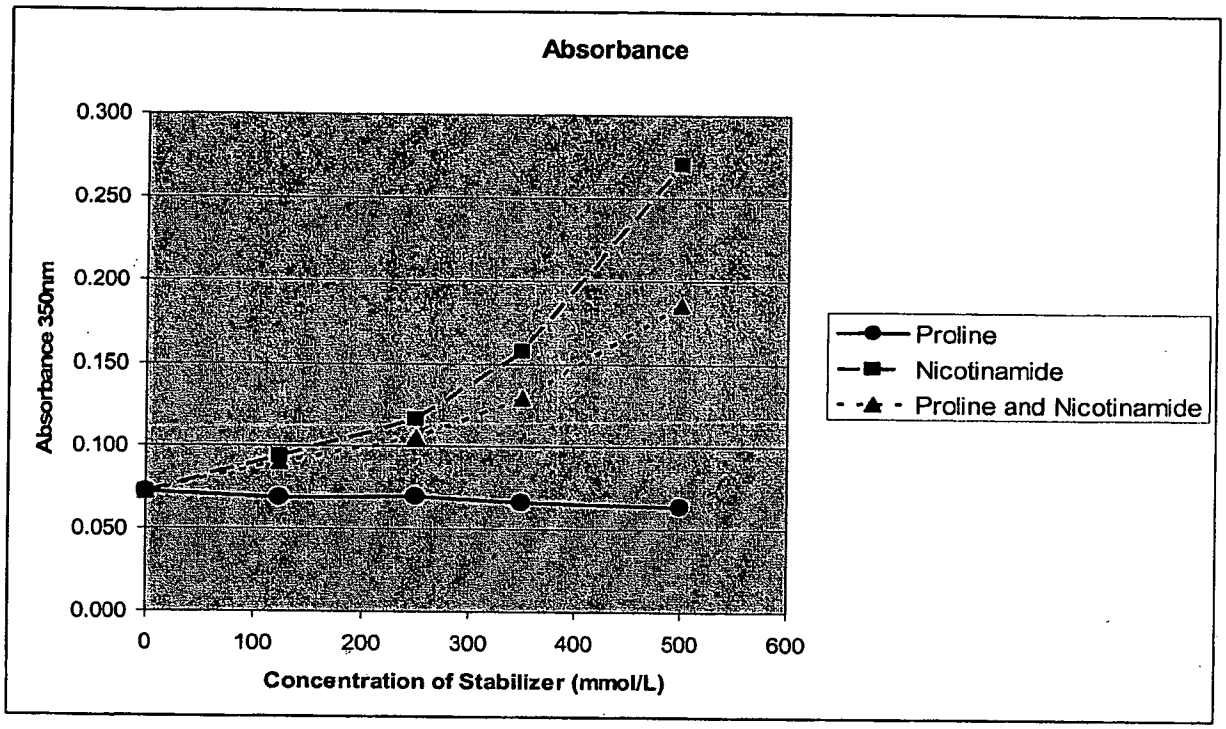


Figure 2: Absorbance at 350 nm (yellowish colouring) of 10% IgG solutions after storage at 40°C for 30 days.



11. The results showed that 10% IgG formulations with proline alone had lower percentage of aggregates and less degree of coloring as compared to the formulations with nicotinamide alone or the formulations with both proline and nicotinamide. Based on my education and experience, these results are unexpected in view of the teachings of the '736 patent, which taught using proline and nicotinamide.

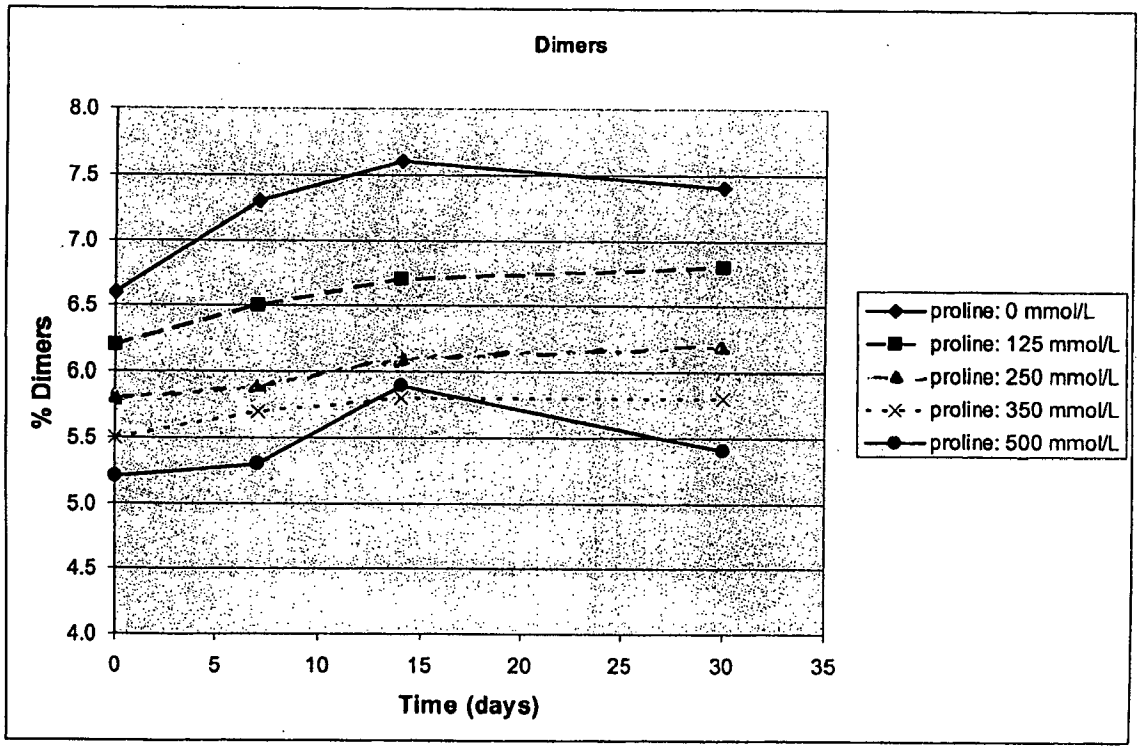
Dimer formation of IgG solutions formulated with proline at different final concentrations

12. 10% IgG solutions were prepared as described above and formulated with 0,125, 250, 350 and 500 mmol/L proline at a pH of 4.8 ± 0.2. The formulations were then incubated at 40°C in the dark. After indicated times of incubation, my laboratory used size exclusion HPLC with a TSK 3000SW column to measure the content of IgG dimers in the solution. The results are shown in Table 2 and Figure 3.

Table 2: IgG-dimer formation in 10% IgG solutions during storage at 40°C

Proline (mmol/L)	Dimer content (%)			
	Day 0	Day 7	Day 14	Day30
0	6.6	7.3	7.6	7.4
125	6.2	6.5	6.7	6.8
250	5.8	5.9	6.1	6.2
350	5.5	5.7	5.8	5.8
500	5.2	5.3	5.9	5.4

Figure 3: Dimer formation in 10% IgG solutions during storage at 40°C for 30 days



13. The results indicated that the dimer content or the dimer formation was significantly reduced in IgG-solutions formulated with proline alone. A satisfying

reduction of dimer content was achieved between 200mM and 400mM proline. However, proline concentration at 500 mM did not seem to result in consistent and uniform reduction of dimer formation over time. In addition, to the extent that the '736 patent merely discloses a proline concentration of up to 200 mM, I believe a skilled artisan would have no reason nor motivation to increase the proline concentration beyond 200 mM because it would increase the cost of the preparation and the osmolarity of the solution, both of which could lead to undesirable outcomes for clinical applications. Accordingly, I believe that the claimed invention is novel and unexpected in view of the concentration range suggested by the '736 patent.

14. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Dated: 28.01.09 By: R. Bolli

Reinhard Bolli